Cumulative Experiences and Heterogeneity Affect Fish Survival: Examples from a model species (*Poecilia reticulata*) and salmonid species (*Oncorhynchus* spp.)

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Abstract

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Jennifer Lam-Anh Gosselin

Chair of Supervisory Committee:
Research Professor James J. Anderson
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Current survival can be affected by the previous conditions experienced including food availability, stressful environmental conditions, and behavioral interactions with other individuals. In this dissertation, I explored relationships between various types of prior experiences and current survival capacities of fishes in experiments that help answer broad ecological questions and assess mitigation strategies in applied sciences. In Chapter 2, I determined that competition-induced heterogeneity among individuals can increase survival at the population level by challenging a model fish species, guppies (*Poecilia reticulata*), that had been reared at various levels of food availabilities and presence or absence of competition, to increased water temperature and in the absence of food. In Chapter 3, I revealed that two types of lethal stress occurring at different time scales can result in episodes of selection and a step-like pattern in the survival curves of juvenile hatchery rainbow trout (*Oncorhynchus mykiss*). In Chapter 4, the mitigation strategy of the Juvenile Fish Transportation Program in the Federal Columbia River Power System (Washington and Oregon) was assessed by examining the relative survival capacities (or vitality) of run-of-river and barged hatchery spring/summer Chinook salmon (*O. tshawytscha*) collected immediately after passage through the hydropower system. In general, the water temperature previously experienced was different between ROR and barged fish, and

showed the greatest influence on survival capacities. In Chapter 5, I determined critical thresholds of simple physical condition indices in Coho salmon (*O. kisutch*) and investigated whether skewed distributions of condition indices for these fish can be used as an indicator of a population experiencing lethal stressors (increased water temperature and absence of food). Although survival can be complex to investigate because of the many processes occurring across different dimensions (e.g. temporal scale, heterogeneity, and skewness) and different biological levels (e.g. subcellular, cellular, individual, and population), my challenge experiments were a relatively simple method to investigate processes underlying survival patterns, and helped show the importance of cumulative effects on survival.

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DEDICATION

To my loving husband, Kevin.

Chapter 1

Introduction

How does the survival of an individual depend on its cumulative experiences? And how is survival at the population level affected by overall heterogeneity among individuals? These are the two main questions I posed in this dissertation inspired by the relationships observed in fishes between growth and size-selective predation (Sogard 1997), lipid deposition and overwinter survival (Biro et al. 2004, Finstad et al. 2004), and type of passage through a hydropower system and disease susceptibility (Arkoosh et al. 2006), to name a few. Only relatively recently have studies to quantify and model the cumulative effects on cross-life stage survival occurred (Anderson et al. 2008, Healey 2011). Thus, much remains unknown about how the underlying processes in cumulative effects influences survival at the individual and population levels. Understanding these types of relationships has been considered particularly important in fishes because they often have life stages across different environments with various natural selection processes (Healey 2011).

Quantifying survival and its link to underlying processes has proven to be challenging. It is thus convenient, and perhaps necessary, to consider survival abstractly in terms of vitality (or survival capacity; Anderson 1992, Anderson 2000). I term "survival capacity" as an overall capacity of an individual to remain alive. An individual that is disease-free, that has high energetic reserves, and that is capable of withstanding many environmental stressors can be considered to be an individual with high survival capacity. In contrast, an individual that is diseased, that has depleted its energetic reserves, and that shows signs of stress at the physiological and biomolecular levels can be considered to be an individual with low survival capacity. Although one can never measure the absolute amount of survival capacity in an

individual, one can record relative measures of it through vitality modeling and challenge studies with standardized stressors. Based on a general concept of how cumulative individual experiences and how heterogeneity among individuals affect survival capacity (Figure 1.1), I examined some underlying mechanisms including food competition, depletion of energetic reserves, and temperature-related processes.

Temperature, particularly at a time of climate change, plays a role in the activities of all living organisms and thus their survival (Brett 1952, 1956, Walther et al. 2002, Roessig et al. 2004). The effects of temperature on survival may be best studied in aquatic poikilotherms which have little or no internal control of their body temperatures, and thus absorb the temperature of the surrounding highly conductive water (Paladino et al. 1980). The effects of increased water temperature on fish include acclimation and tolerance processes (Whittow 1970, Chavin 1973, Beitinger et al. 2000), modification of foraging and migration behaviors, and changes in geographical distributions (Roessig et al. 2004). In my dissertation, I examined research questions related to survival by relatively simple challenge experiments of increased water temperature with salmon (*Oncorhynchus* spp.), one stock of which is currently listed under the Endangered Species Act (NMFS), and with guppies (*Poecilia reticulata*), a model species. Furthermore, the range of tolerances of fish in temperature challenge experiments can be an indicator of the fish's current condition and survival capacity which in turn may be affected by cumulative experiences (Brett 1952, Hutchinson 1976, Wedemeyer et al. 1990).

In this dissertation, I touch upon four main topics related to the effects of cumulative experiences and heterogeneity on survival capacities. I begin on a positive note, and present a fundamental way to increase survival of a population (Chapter 2). I then present a study that supports the importance of cross-disciplinary examinations of survival, spanning from life stages to underlying biological mechanisms (Chapter 3). At the heart of the dissertation is an

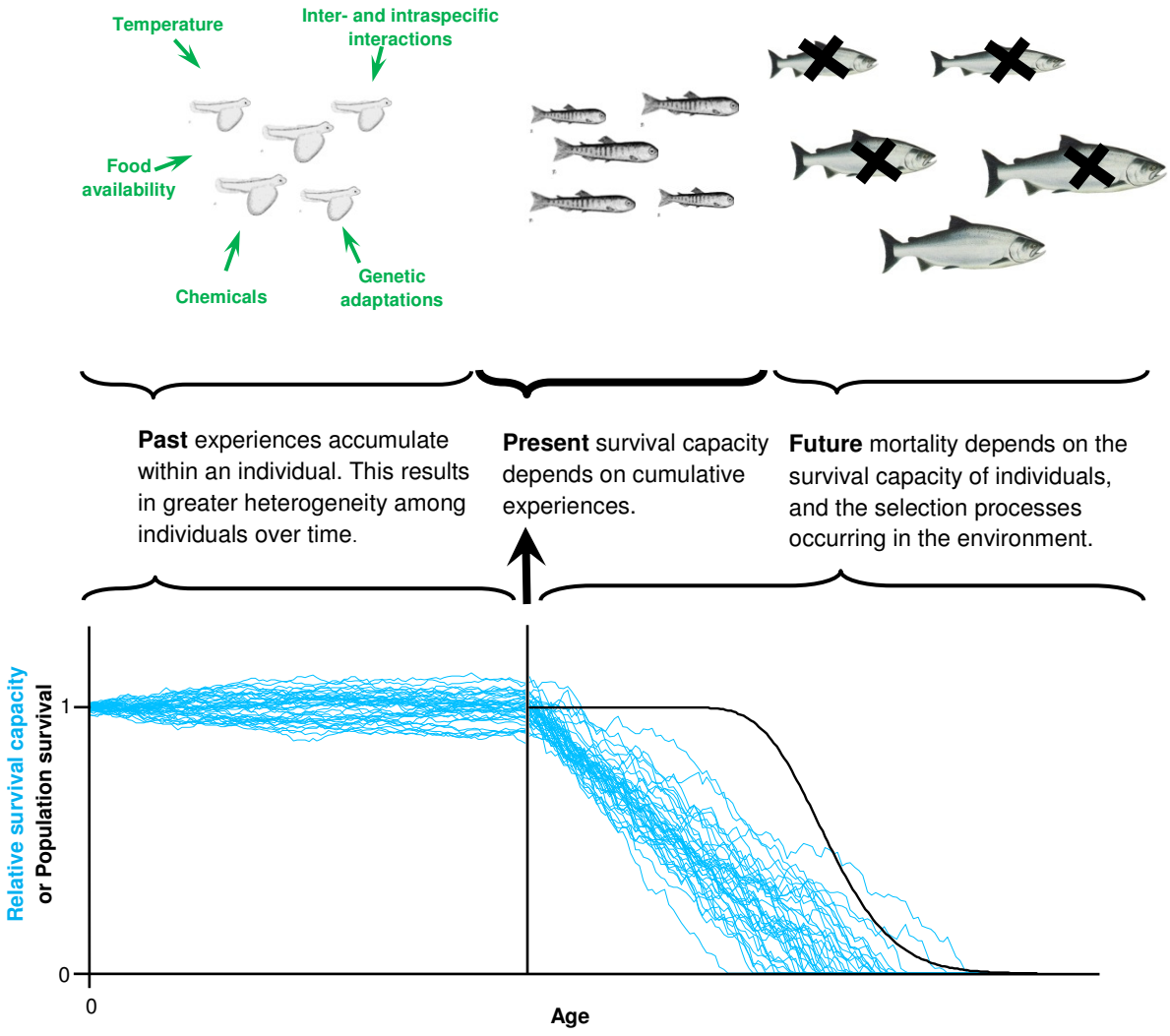

Figure 1.1. Conceptual diagram of how cumulative experiences and heterogeneity among individuals affect survival capacity (blue lines) and population survival (black curve).

investigation of the effects of an anthropogenically modified habitat and a mitigation strategy on the survival capacity of endangered salmon stock (Chapter 4). Finally, I assessed a hypothesized phenomenon underlying population mortality and a potential way to determine the degree of population mortality and fish condition based on snapshot data of simple physical indices of fish experiencing lethal stress (Chapter 5).

In Chapter 2, I investigated whether competition-induced heterogeneity among individuals can buffer against mortality of a cohort (or population) in a challenge experiment with guppies. Although variation among individuals naturally occurs, its benefits to population survival have only recently been identified at the level of life history traits (Schindler et al. 2010), and the focus has more generally been at the broader scale of species biodiversity and ecosystem functioning (Simberloff 1999, Cottingham et al. 2001, Petchey and Gaston 2002). At the population level, positive relationships between mass (or size) and survival (Rice et al. 1993, Sogard 1997), and between resource competition and variability among individuals in their sizes (Weiner 1986, Blanckenhorn 2006, Huss et al. 2008) have often been found. However the interrelationship between the three (competition, mass or size, and survival) have not been well studied.

Organisms encounter many stressors throughout their lifetime, and these events can result in episodes of natural selection (or bottlenecks) (Cunjak et al. 1998, Armstrong et al. 2003). Although survival curves and episodes of natural selection are two common concepts in ecology, studies that incorporate both are relatively rare (Yashin et al. 2002, Wu et al. 2006). The ability of organisms to acclimate to thermal stress is necessary for survival during a time of climate change (Hoegh-Guldberg 1999, Deutsch et al. 2008). I predict that phenotypic differences in thermoregulatory ability can be represented by distinct patterns in survival curves. In a simple challenge experiment involving two stressors (increased water temperature and absence of food resources) hypothesized to affect fish survival over two different time scales (Paladino et al. 1980, Biro et al. 2004), I investigated the step-like pattern in the survival of juvenile rainbow trout (*Oncorhynchus mykiss*) and juvenile spring/summer Chinook salmon from the Columbia River (*O. tshawytscha*) in Chapter 3. In this study, I also test whether the loss of equilibrium (LOE) is a useful surrogate endpoint for mortality of fish in challenge experiments. Although

average and median times to LOE have been compared to those of mortality, the pattern of time difference between occurrences of LOE and mortality for a whole survival curve has not been determined.

In light of the importance of cumulative experiences to survival, I investigated the seasonal patterns of survival capacity in a Pacific salmon stock listed under the Endangered Species Act (ESA; NMFS 2011) after its passage through a hydropower system. During and after the construction of Federal Columbia River Power System (Oregon and Washington, USA), salmon stocks dramatically declined (Raymond 1988, Williams 2008), instigating numerous efforts in the development and application of mitigation strategies. One mitigation strategy is the Juvenile Fish Transportation Program, implemented by the U.S. Army Corps of Engineers, which involves barging smolts from three of the most upstream dams (Lower Granite Dam, Little Goose Dam, and Lower Monumental Dam) to below the most downstream dam (Bonneville Dam) of the hydropower system. Although survival rates through the hydropower system have doubled from approximately 50% (run-of-river migration) to 98% (barge transportation), indirect mortality is occurring post-Bonneville Dam and at different rates for fish of the two passage types (run-of-river and barged fish) (Anderson et al. 2005, Williams et al. 2005). In Chapter 4, I investigated indices of survival capacity of run-of-river and barged fish after passage through the hydropower system in challenge experiments at increased water temperature and in the absence of food (Subchapter 4A), by quantifying time to loss of equilibrium in an anesthetic dose of tricaine methanesulfonate (Subchapter 4B), and by quantifying proximate compositions such as total body fat and total body protein (Subchapter 4C).

Determining how much mortality has occurred in a fish population can be challenging because dead individuals are generally not detected (Dutil and Lambert 2000) and long-term datasets require great efforts. In Chapter 5, I explore a way of using snapshot data to determine

the degree of mortality and fish condition at the population level. It has been hypothesized that the statistical distribution of a fish condition index or survival capacity changes from a normal distribution to a skewed distribution as selection occurs (Anderson 1992, Anderson 2000, Rand et al. 2006). Mortality of poor quality fish results in the truncation of one side of the distribution and thereby increases skewness. By using simple physical indices of fish condition, some of which are indicative of energy density (Hartman and Brandt 1995), I examined the temporal changes in the distributions of fish condition indices in juvenile Coho salmon (*O. kisutch*) under lethal stress.

Overall, in my dissertation, I investigate how abstract (e.g. vitality) and concrete (e.g. time to LOE, proximate composition, physical measures) indices can help estimate the conditions and survival capacities of fishes. In the process, I have produced work that further emphasizes the importance of uncovering survival patterns from the environmental conditions that fish have cumulatively experienced as well as the biological processes within a fish acclimating to changing environmental conditions. Furthermore, to better understand indices and underlying processes of survival, I purposely delved into the dimensions of heterogeneity among individuals and how fish conditions change over time. These themes of survival indices, underlying processes, cumulative experiences and heterogeneity across time scales are present in all four research chapters that cover: how survival can be increased by heterogeneity among individuals (Chapter 2), how step-like patterns of selection can reflect episodes of selection and phenotypic variation (Chapter 3), whether loss of equilibrium can be used as a surrogate endpoint for mortality (Chapter 3), the seasonal patterns and possible causes of relative survival capacity of run-of-river and barged fish after hydrosystem passage (Chapter 4), and whether skewed distributions of fish condition indices are indicative of a population under stress (Chapter 5).

Chapter 2

Resource Competition Induces Heterogeneity and Beneficial Population Selection

Abstract

Increased survival due to increased mean growth has often been demonstrated in fish populations, but the effects of growth variability on survival remains elusive. In this study, I examined the effect of food competition on variability in body masses and survival capacity. In a two-stage experimental protocol, four groups of juvenile guppies (*Poecilia reticulata*) were first reared at differing amounts of food (low, moderate or high) and degrees of foraging competition (presence or absence) for 21 days, and then challenged with increased water temperature and an absence of food resources. By fitting the survival patterns obtained in the challenge stage with a vitality model that characterizes population survival capacity, I was able to infer the effect of food-availability on mass and how competition altered heterogeneity in population vitality. The model-derived group means and coefficients of variation in vitality obtained from the challenge-stage survival curves were linearly related to the means and coefficients of variation of body mass measured in the groups at the end of the treatment stage. My results demonstrate that feeding competition increases population heterogeneity in both mass and survival capacity (vitality). I suggest that under limited resources, high-quality individuals in a population can experience higher survival with greater competition-induced heterogeneity. Uses of challenge studies coupled with vitality modeling are discussed in the context of examining dynamics of population selection in hatchery and wild fish.

Introduction

Unraveling the mechanisms underlying the viability of populations is central to conservation ecology. Whether the motivation is for maintaining biological integrity (Larkin 1996, Callicott et al. 1999, Niemi and McDonald 2004), sustaining the economy (Brklacich et al. 1991, Murawski 2000, Hilborn et al. 2003), or generating scientific knowledge, the strive to improve population survival continues for many species around the world. Common strategies in conservation ecology focus on habitat restoration (Boyce and McDonald 1999, Lytle and Poff 2004, Lake et al. 2007), protected areas (Saunders et al. 2002), and the eradication or alleviation of predation pressure (Myers et al. 2000). One process of promoting population viability that remains largely unexplored is intraspecific competition because it is generally examined at the level of individuals. In this chapter, I show how intraspecific competition can buffer against mortality at the level of the population.

Increased variability in size caused by resource competition has been demonstrated in plants and animals (Rubenstein 1981, Weiner 1986, Blanckenhorn 2006, Peacor et al. 2007, Huss et al. 2008). Resource competition can exacerbate the differences in sizes among individuals, upon which larger individuals disproportionately consume more and grow bigger than smaller individuals (Weiner 1990, Berger et al. 2008, Yonekura et al. 2009, Nakayama and Fuiman 2010). This phenomenon has been termed asymmetric competition, and has been observed in a variety of organisms. In plants that are competing for light, larger-sized individuals can acquire more than their share in proportion to their size of shoots, and suppress the growth of smaller-sized individuals by overshadowing them (Weiner 1990). In fishes, small-sized individuals in direct behavioral competition (or interference competition) consume proportionally less prey in relation to their body size than large-sized fish (Nakayama and Fuiman 2010). As the intensity of competition increases, the degree of size variability also increases (Rubenstein 1981, Sogard and

Olla 2000, Huss et al. 2007, 2008). Overall, competition has been established as a force of increased variability among individuals; however no studies have demonstrated competition-induced effects on cohort or population survival.

Size and growth have been well established as important factors of survival (Rice et al. 1993, Letcher et al. 1996a, Sogard 1997, Moss et al. 2005, Duffy and Beauchamp 2011), and thus asymmetric competition-induced changes in size will also likely affect survival. Two size-related mechanisms of mortality are metabolic requirements and size-selective predation. Overwintering fish require sufficient energetic reserves to overcome months of limited food resources (Sogard and Olla 2000, Biro et al. 2004). Many fish species undergo a bottleneck in their first winter (Shuter et al. 1980, Conover and Present 1990, Olson 1996, Klemetsen et al. 2003). Larval and juvenile fish compete to maximize individual growth in their first growing season to reach a minimum threshold size (Houde 1997), weight (Cowan et al. 2000), or energetic content (Biro et al. 2004) for overwinter survival. Also, prey size is closely linked to their predator vulnerability (Galbrait 1967, Parker 1971, Litvak and Leggett 1992, Persson and De Roos 2007). The gape size of predators limits the size of prey they can consume. Larger-sized fish thereby experience lower predation risk than smaller-sized fish. Under these two size-related mechanistic processes, increasing the pressure of intraspecific competition, and hence the quality of select individuals, could increase population survival. The resources not consumed by small fish destined for overwinter starvation or predation may enhance large fish survival and consequently the survival of the population.

In the present study, I examine whether food competition increases overall heterogeneity among individuals, and thereby generates a proportion of individuals that exhibit greater quality and survival than had they not experienced competition. I conducted an experimental mesocosm experiment coupled with vitality modeling (or survival capacity modeling). The experiment

involved a treatment stage in which fish were reared at different levels of food availability and food competition in guppies (*Poecilia reticulata*), followed by observations of survival in a challenge of increased water temperature and starvation. The body masses and model-derived (Li and Anderson 2009) survival patterns during the challenge stage revealed that: 1) initial body mass was positively related to survival capacity, 2) as the variability in initial body mass increased, so did the heterogeneity in survival capacity, and 3) food competition provided a buffering effect against mortality for the highest quality fish in the group.

Methods

Laboratory Experiment

The guppies (*Poecilia reticulata*) tested in this study were born in a laboratory at the University of Washington. The treatment stage included different combinations of food availabilities and food resource competition occurred from birth to 21 days of age, and was followed by a challenge test stage of increased water temperature and starvation (Figure 2.1). This research study was approved by the University of Washington Institutional Animal Care and Use Committee under protocol #3382-04. Water quality monitoring and treatment, lighting conditions, and shelter materials in the aquatic tanks are described in the Appendix A.

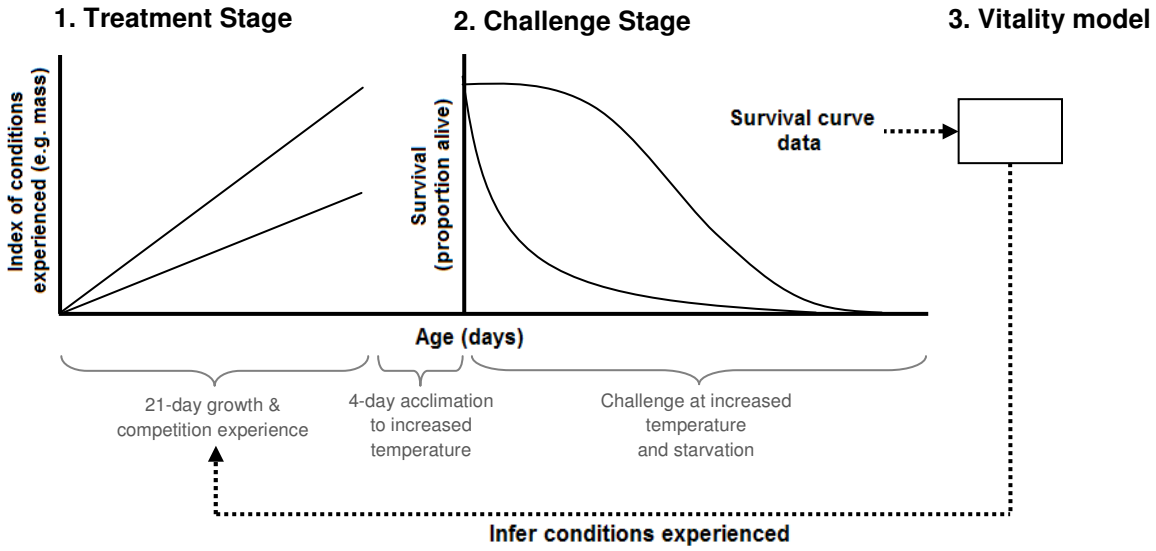

Figure 2.1. Conceptual diagram of fish challenge study coupled with vitality modeling to infer previous conditions experienced.

Parental guppies of test individuals

The parental guppies used to produce the test individuals were from the Guanapo population, and were second generation laboratory-reared guppies from wild samples collected in Trinidad (donated by Professor Cameron Ghalambor, Colorado State University; N=411). Guppies from the Guanapo population are known to originate from an environment with relatively high intensity predation pressure and thus produce large sized broods ideal for this study. The parental guppies were maintained from 9 December 2009 to 13 March 2010 in a recirculating aquarium system of eighty 1-L tanks containing 22°C water. Each of the 80 tanks contained 4 to 6 guppies. They were fed twice a day with Tetrafin dried fish flakes or live *Artemia* brine shrimp nauplii (hereafter termed brine shrimp) hatched in the laboratory. When the target number of offspring produced for testing was reached, the parental guppies were euthanized with a lethal dose of tricaine methanesulfonate (MS-222; 250 mg/L buffered to 7.0 with $NaHCO_3$).

Treatment stage of test guppies

I looked for newly produced offspring every morning and every evening in the parental tanks. As soon as offspring were detected, they were transferred to 8-L static tanks. At the end of the day, offspring born throughout the day were weighed to the nearest milligram in batches because each individual was too small to weigh accurately on the balance. They were then introduced to one of four treatment groups: low food availability with competition (L.C), moderate food availability with competition (M.C), moderate food availability without competition (M.NC), and high food availability without competition (H.NC). These different levels of food availability and competition were selected to allow comparisons of average masses within the same level of competition (L.C vs. M.C; M.NC vs. H.NC) and a comparison of presence and absence of competition with a similar average masses (M.C vs. M.NC) in a restricted number of treatment groups. Because only a limited number of offspring were born each day, guppies born within four days, mostly two days, of each other were grouped into the same replicate treatment group. The number of replicate tanks for the L.C, M.C, M.NC, and H.NC treatment groups were 5, 5, 6, and 5 respectively, and the total number of test guppies were 169, 188, 180, and 181 respectively.

During the 21-day treatment stage, the offspring were fed brine shrimp following a schedule that was expected to result in low, moderate, and high masses, with or without food resource competition. Guppies in the NC treatment groups were fed to satiation once a day for the M.NC group and twice a day for the H.NC group. The amount of food approximated for satiation was observed in two guppies by counting the number of brine shrimp each guppy consumed until satiated. Guppies in the competition treatment groups were fed twice a day an amount less than that estimated for satiation in order to promote food competition. At each feeding, the fish in the

L.C treatment group were fed 10% of the satiation amount and the fish in M.C treatment group were fed 40% of the satiation amount. When feeding brine shrimp to the guppies, the amount was first quantified as the number of individuals and later converted to mass (Appendix A). At the end of the treatment stage, when the guppies were 21 days of age, wet body mass was recorded for each individual.

Challenge stage of test guppies

After the treatment stage, the guppies were introduced to a challenge of increased water temperature and starvation. A target temperature of 33°C was chosen based on published literature of critical thermal maxima in related species (Chung 2001) and pilot trials. The acclimation period for the gradual increase in water temperature from 22°C to 33°C occurred over 4 days (Appendix A). During the challenge stage, guppies were observed at least three times a day for loss of equilibrium and mortality. If loss of equilibrium was observed, guppies were euthanized in a lethal dose of MS-222 (250 mg/L buffered to 7.0 with $NaHCO_3$) as a surrogate endpoint to reduce nociception in the test animals. The time at which death or loss of equilibrium occurred, and wet body mass were recorded for each guppy.

Data Analysis

General estimates of fish condition from body mass

The average wet body mass at birth, after the treatment stage (at approximately 21 days of age), and at the endpoint (time at death or loss of equilibrium) were calculated. Not all individuals challenged were weighed because of time constraints and incomplete retrieval of dead guppies. The standard deviation and coefficient of variation were determined only for masses after the treatment stage and at the endpoint because masses at birth were bulk weighed. The

masses helped to determine the degree of similarity across all test animals at birth, as well as the degree of variability within each treatment group after the treatment stage and at endpoints during the challenge stage.

Survival and heterogeneity estimated by the vitality model

Measures of survival and heterogeneity for each of the four treatment groups were estimated by a vitality model (Li and Anderson 2009). Vitality (v) is an abstract measure of survival capacity that is thought to decrease with an accumulation of physiological and genetic damage throughout one's lifespan (Anderson 2000, Anderson et al. 2008). Over one's lifespan, the decline in vitality to zero is characterized as a rate of change in vitality by the following stochastic differential equation:

$$\frac{dv}{dt} = -\rho + \sigma\varepsilon_t \qquad \text{(Eq. 1)}$$

where ρ is the average rate of vitality loss, σ is the variability in the rate of vitality loss (also termed evolving heterogeneity), and ε is the function of stochastic variability in the rate over time t. The probability of when the vitality of an individual randomly drifts to zero occurs as the continuous-time stochastic Wiener process. Also, the initial distribution of vitality (termed initial heterogeneity) is characterized by a standard deviation τ (see Li and Anderson 2009 for detailed derivations of the model). Theoretically, the vitality parameters ρ, σ, and τ are constant throughout a lifetime. In practice, these parameters can be normalized by an estimate of the mean initial vitality of the population ($\bar{v}_0$) to yield r, s, and u respectively. Thus, differences in survival rates within and across populations are due to differences in v_o. Figure 2.2A graphically depicts the vitality parameters with the individual vitality trajectories represented by the green lines, r by the red line, s by the blue shaded area, and u by the purple curve. Furthermore, the vitality density

characterizes the population of individuals over time, and the shape of its distribution changes similar to the advection-diffusion process as it progresses from its initial vitality (v_o) to zero over time (black curves in Figure 2.2B). As individuals reach the zero boundary, they die from complete loss of vitality and are removed from the population. The vitality density (i.e. area under the curve) is the proportion of individuals remaining alive. Integration of the vitality density gives the vitality-based survival curve (black curve in Figure 2.2A). Extrinsic mortality can also occur which, unlike the vitality-based survival function, is a process independent of an individual's age. Survival is thus a combination of the vitality-based and the extrinsic-based survival functions. In summary, parameters of the vitality model include t is time (units of t), Φ is a cumulative normal distribution, r is the normalized rate of vitality loss (units of t^{-1}), s is the normalized variability in the rate of vitality loss (units of $t^{1/2}$), u is the coefficient of variation of the initial vitality distribution (when normalized, it is a Gaussian distribution with a unit mean of one and variance u^2), and k (units of t^{-1}) is the extrinsic mortality rate characterized by a Poisson process. Maximum likelihood estimates of the four vitality parameters (r, s, u, and k), their standard errors, and the goodness-of-fit can be determined by an algorithm in the software program R and is available at http://www.cbr.washington.edu/vitality/.

A survival curve was fitted for each treatment group. Replicate tanks within each treatment group were combined to produce a survival curve because the number of individuals from each replicate tank was too small to robustly fit with the model. In the most stressed treatment group (L.C group), twelve guppies died during the increase in water temperature before the onset of the challenge stage and were excluded from the survival curve analysis. One replicate tank in the H.NC had a temporary increase in water temperature 3°C beyond the target temperature and was also excluded from further analysis. Extrinsic mortality caused by factors such as disease or lethal water quality was assumed to be absent in all the other remaining tanks

and thus I limited the parameter estimation for k by maximum likelihood to range from 0 to 0.004. Estimates of s for each treatment group i were determined theoretically (Eq. 2) based on r_i values in an initial run of the model determined by maximum likelihood estimation. In the final run of the model, the range specified was $s_i \pm 0.01$. Furthermore, an estimate of the normalized vitality (v_i) for each treatment group was approximated theoretically:

$$\frac{r_o}{r_i} = \frac{s_o}{s_i} = \frac{v_i}{v_o} \qquad \text{(Eq. 2)}$$

where subscript i refers to the four different treatment groups, and subscript o refers to individuals at $t = 0$ of the challenge had they not experienced any treatment of diminished food availabilities or food competition. The value for v_o was approximated by the v of the H.NC group after the treatment stage (i.e. beginning of challenge stage).

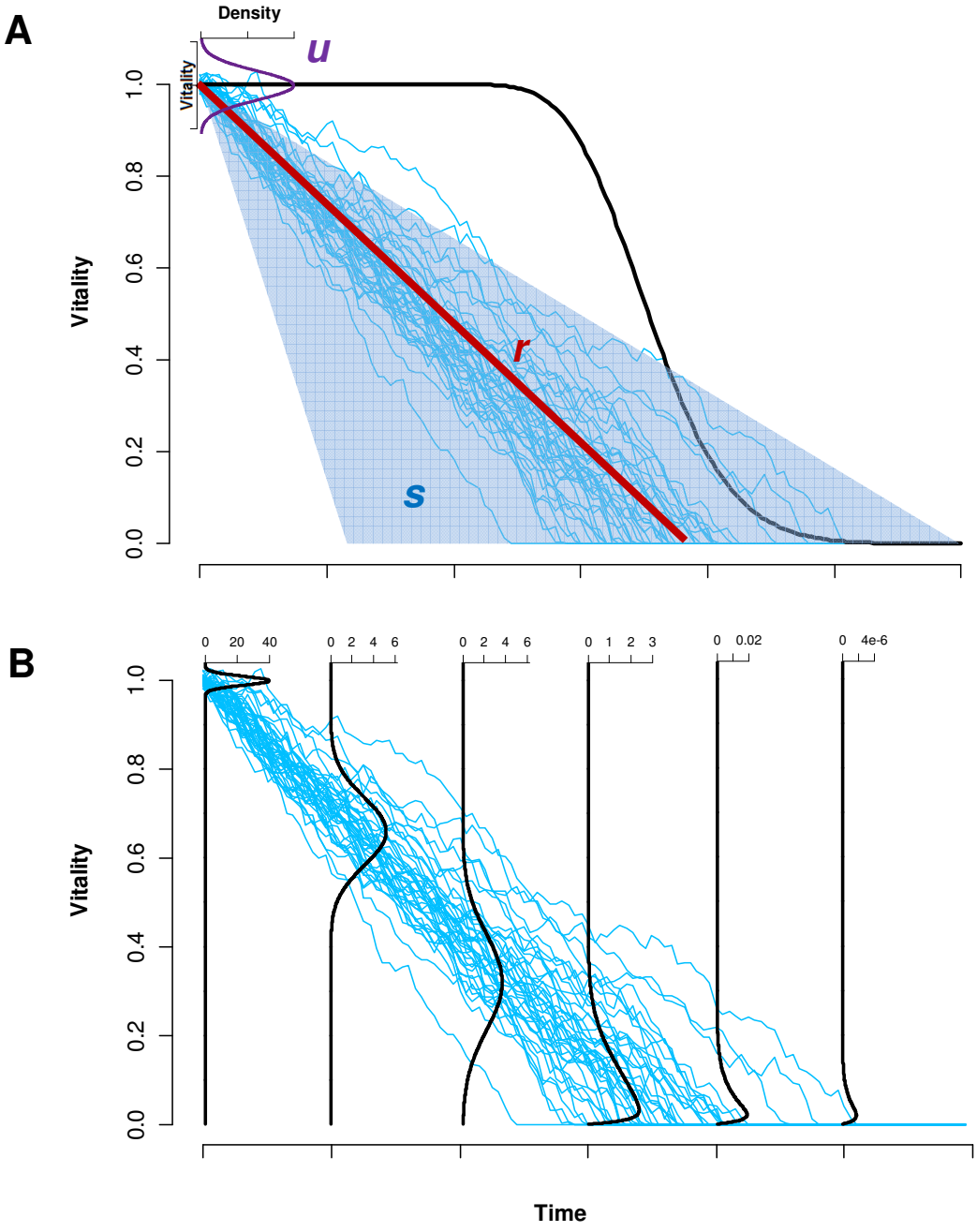

Figure 2.2. (A) Visual description of Li and Anderson's (2009) vitality parameters: average rate of vitality loss (r; units of t^{-1}); variability in the rate of vitality loss or evolving heterogeneity (s; units of $t^{1/2}$), and initial heterogeneity quantified as a coefficient of variation, (u; unitless). (B) Conceptual diagram of vitality density (black lines) relative to individual vitality trajectories (blue lines).

To examine the various relationships between competition, mass variability, and survival, we examined various linear regressions between vitality parameters and masses. Linear relationships were determined between: 1) survival during the challenge stage and body mass after the treatment stage, 2) vitality at onset of challenge and body mass after the treatment stage, 3) initial heterogeneity at the onset of the challenge and CV of mass after the treatment stage, and 4) mass at time of challenge mortality and mass after the treatment stage. To visualize the evolution of the vitality of each treatment group over time, probability density functions were graphed for values on various days throughout the challenge. Finally, to better distinguish the effects of mass and heterogeneity on survival of the groups with moderate food availability, I simulated two survival curves assuming different *r* parameters while keeping the heterogeneity parameters constant, and simulated two other survival curves assuming different heterogeneity parameters while keeping *r* constant. In the first case, I compared the simulated groups which either had an *r* parameter of M.NC (simulated "higher mass" group) or one of M.C (simulated "lower mass" group) while holding the heterogeneity parameters constant. In the other case, I compared one group which had the *s* and *u* heterogeneity parameters estimated from M.NC (simulated "lower heterogeneity" group) to a group which had heterogeneity parameters estimated from M.C (simulated "higher heterogeneity" group) while holding the *r* parameter constant with the value estimated for the M.NC group.

Results

The masses of guppies at birth were statistically equivalent across all four treatment groups (Table 2.1; weighted ANOVA, $F_{3,19} = 1.468$; $p = 0.255$. The feeding schedule during treatments was successful in generating guppies of low, moderate, and high body masses (Table 2.1; Figure 2.3; ANOVA, $F_{3,665} = 1479.7$, $p < 0.001$). The groups with moderate food availability

were most similar in masses after the treatment stage, but the M.NC group still had a significantly higher average mass than the M.C group (two-way T-test, $t = -9.93$, $df = 363.471$, $p < 0.001$). The coefficients of variation (CV) of mass were lowest in the two groups without competition, moderate in the M.C group and highest in the L.C group (Table 2.1).

The survival curve for each treatment group was successfully fitted by the vitality model and had a statistically significant goodness-of-fit (Table 2.1; Figure 2.4A). Overall, survival was lowest in the group with low food availability and highest in the group with high food availability. In the groups with moderate food availability, lower survival occurred earlier in the challenge in the competition group. Their survivals were then approximately the same starting at day 12 of the challenge. This late-occurring equivalence in survival occurred despite the overall lower average mass (two-way T-test, $t = -9.93$, $df = 363.471$, $p < 0.001$) and overall lower average time to mortality (Table 2.1; two-way T-test, $t = -2.03$, $df = 364.26$, $p < 0.001$) of the M.C individuals relative to M.NC individuals. Similarities and differences in the survival curves for the groups with moderate food availability can be more easily perceived in Figure 2.4B with the natural log of survival rates. The proportional change in vitality of guppies over the course of the challenge can be viewed by probability density functions (Figure 2.5). Overall, relative vitality was lowest in the group with low food availability and highest in the group high food availability. The vitality density curves for all four treatment groups had their respective greatest average value of relative vitality at $t = 0$. As the challenge progressed, the average relative vitality decreased towards 0 and the spread of vitality increased. The competition groups began the challenge with wider relative vitality density curves than the groups not experiencing competition. In the groups with moderate food availability, a proportion of M.C individuals had lower vitality than M.NC individuals in the early part of the challenge. Thus, the M.C survival curve began decreasing before the M.NC survival curve (Figure 2.4). Also, a proportion of M.C

individuals had greater vitality than M.NC individuals (Figure 2.5). At the onset of the challenge, 45.5% of M.C individuals had greater vitality than M.NC individuals. After 2 days, 5 days, and 12 days, there were respectively 9.1%, 6.5%, and 0.02% of M.C individuals that had higher vitality than M.NC individuals. The patterns in vitality densities over the course of the challenge help explain the patterns in survival curves of the four treatment groups.

To determine how survival, initial vitalities, masses, and measures of heterogeneity were associated, linear relationships were examined. As the average mass after the treatment stage increased, the average time to challenge mortality increased (Figure 2.6A; $r^2 = 0.976$; $p = 0.008$). The initial vitality of guppies in each treatment group was positively related to mass after the treatment stage (Figure 2.6B; $r^2 = 0.978$; $p = 0.007$). Furthermore, as the CV of mass increased, initial heterogeneity also increased (Figure 2.6C; $r^2 = 0.999$, $p < 0.001$). Finally, the average mass at time of challenge mortality was linearly related to the average mass after the treatment stage (Figure 2.6D; $r^2 = 0.999$, $p < 0.001$).

The simulated survival curves helped to isolate the effects of the mass-related vitality loss rates from those of the heterogeneity parameters. In the first case, the "higher mass" group had greater survival than the "lower mass" group at all times of the simulated challenge (Figure 2.7A). In the second case, the "lower heterogeneity" group initially had greater survival than the "higher heterogeneity" group; towards the end of the simulated challenge, this pattern was reversed and the "higher heterogeneity" group survived better than the "lower heterogeneity" group (Figure 2.7B). Thus, increased heterogeneity helps buffer against mortality at the group level.

Table 2.1. Average mass at the start (birth) and end (approx. 21 days old) of the treatment stage. Average survival and vitality parameters of survival curves fitted to mortality data of guppies in the challenge stage of increased water temperature and starvation. The four treatment groups tested were low food availability with competition (L.C), medium food availability with competition (M.C), medium food availability without competition (M.NC), and high food availability without competition (H.NC). The vitality parameters are the rate of vitality loss (r ; units of t^{-1}), evolving heterogeneity (s; units of $t^{1/2}$), initial heterogeneity (u; a coefficient of variation), and extrinsic mortality (k; units of t^{-1}). N represents the number of individuals and n represents the number of bulked groups of individuals. The goodness-of-fit of all four survival curves were statistically significant to the p=0.05 level.

Treatment group	Average mass (mg)			Average time to challenge mortality (days) ± SD (CV)
	At birth	At end of treatment stage ± SD (CV)	At time of challenge mortality ± SD (CV)	
L.C	6.2 N=141; n=6	9.0 ± 2.3 (0.26) N=169	9.0 ± 2.22 (0.25) N=151	2.4 ± 1.8 (0.75) N=157
M.C	6.2 N=158; n=6	15.9 ± 3.4 (0.21) N=188	13.7 ± 3.1 (0.23) N=178	7.5 ± 2.7 (0.36) N=188
M.NC	5.8 N=98; n=6	19.2 ± 2.9 (0.15) N=181	16.3 ± 3.1 (0.19) N=174	8.1 ± 2.4 (0.30) N=181
H.NC	6.3 N=125; N=5	36.0 ± 5.5 (0.15) N=143	27.1 ± 5.0 (0.18) N=142	16.0 ± 3.7 (0.23) N=143

Table 2.1. (cont'd)

Treatment group	Survival Curve Parameters ± SD				Goodness-of-fit p-value
	Vitality r	*Vitality s*	*Vitality u*	*Extrinsic k*	
L.C	0.413 ± 0.068	0.341 ± 0.063	0.388 ± 0.058	0.004 ± 0.081	< 0.001 N=157
M.C	0.134 ± 0.005	0.113 ± 0.021	0.216 ± 0.083	0.003 ± 0.007	0.005 N=188
M.NC	0.127 ± 0.003	0.110 ± 0.007	0.000 ± 0.000	0.000 ± 0.000	0.001 N=181
H.NC	0.063 ± 0.001	0.059 ± 0.004	0.000 ± 0.000	0.001 ± 0.001	< 0.001 N=143

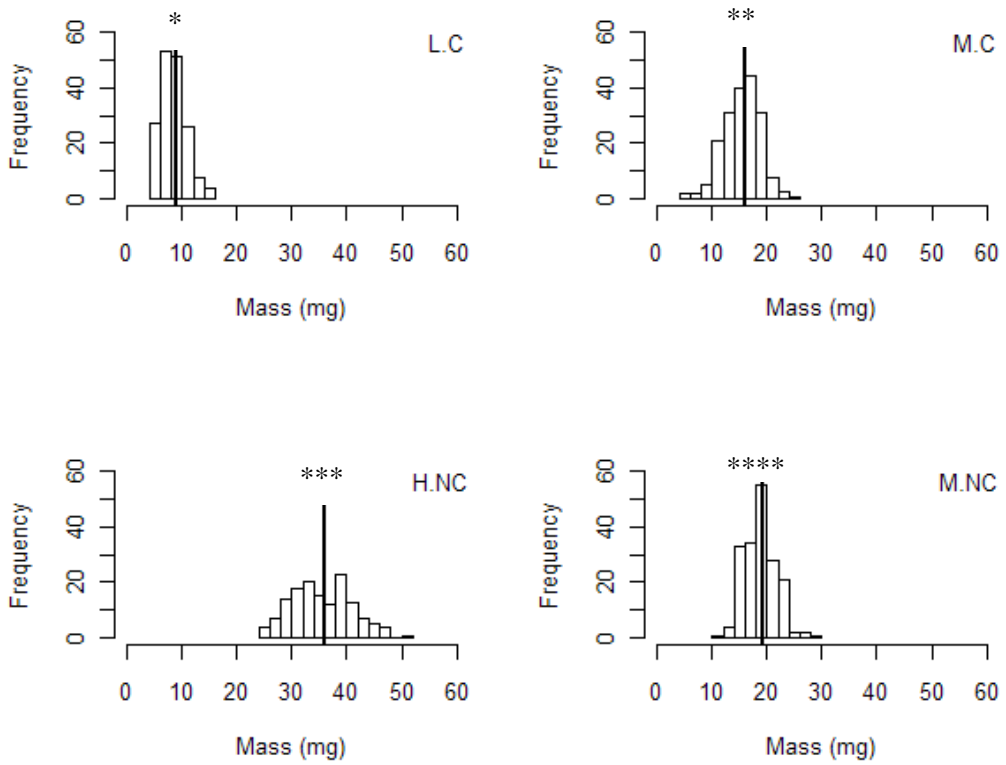

Figure 2.3. Frequency distribution of guppy body masses at the end of the treatment stage. Please see Table 2.1 for complete treatment group names and sample sizes. The thick lines represent the average mass of each treatment group. Different asterisks denote statistical differences with $p < 0.05$.

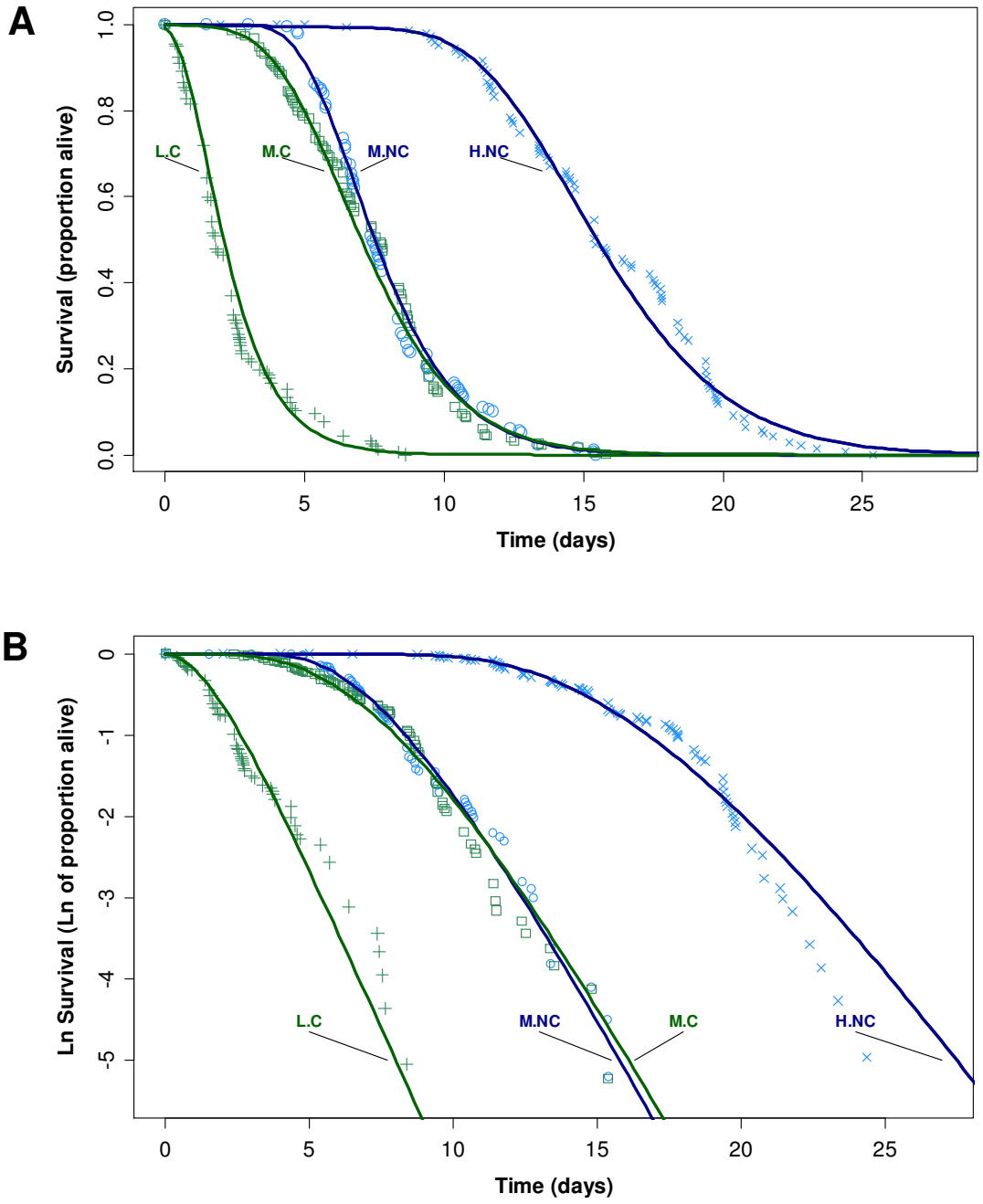

Figure 2.4. (A) Survival curves (lines) fitted by the vitality model to observed mortalities (data points) of guppies in a challenge experiment of increased temperature and starvation; (B) Ln survival (lines) fitted by vitality model to observed mortalities (data points). Please see Table 2.1 for full names and sample sizes of treatment groups.

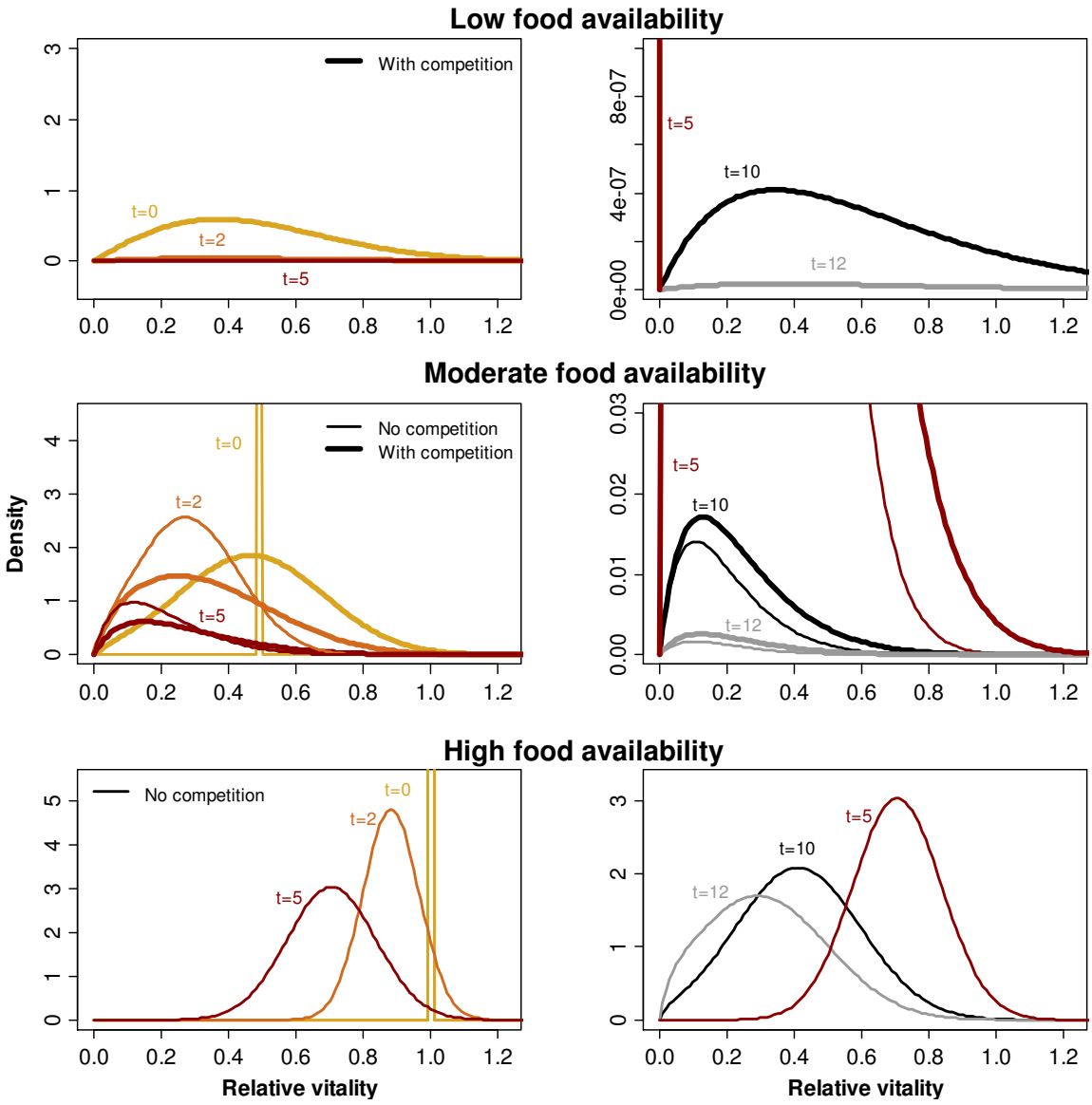

Figure 2.5. Vitality densities of guppies over the course of the challenge, where *t* represents time in days. Thick lines represent groups experiencing competition during the treatment stage, and thin lines represent groups without competition. Left graphs show vitality densities on days 0, 2, and 5; right graphs show vitality densities on days 5, 10, and 12.

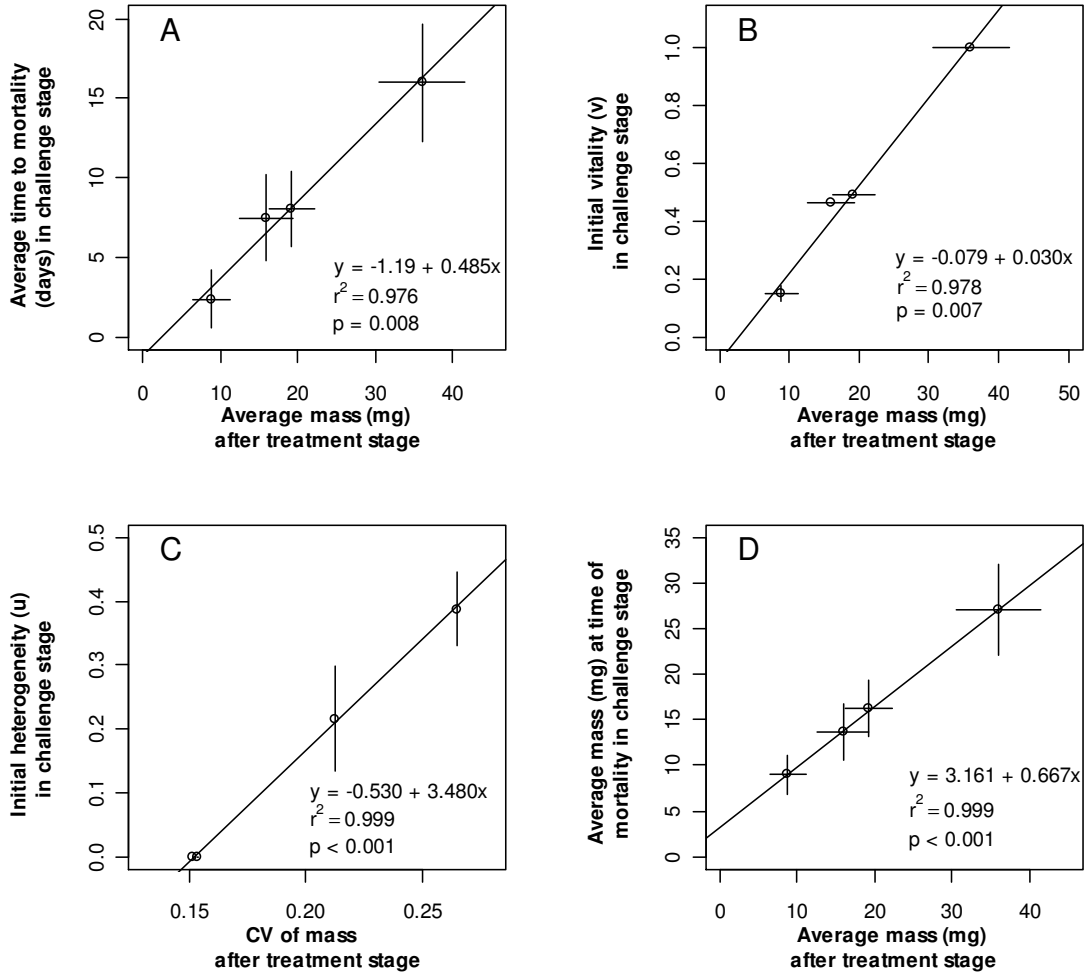

Figure 2.6. Linear relationships between (A) Average time to challenge mortality and average mass after treatment stage; (B) Initial vitality (estimated by vitality loss rate r_i) and average mass after treatment stage; (C) Initial heterogeneity (u) and coefficients of variation (CV) of average mass after treatment stage; (D) Average mass at time of challenge mortality and average mass after treatment stage. The four data points are the four treatment groups tested. Please see Table 2.1 for complete names of treatment groups and sample sizes. Data points at ($u = 0$) for the H.NC and M.NC groups are juxtaposed so that they are both visible. Error bars represent standard deviations.

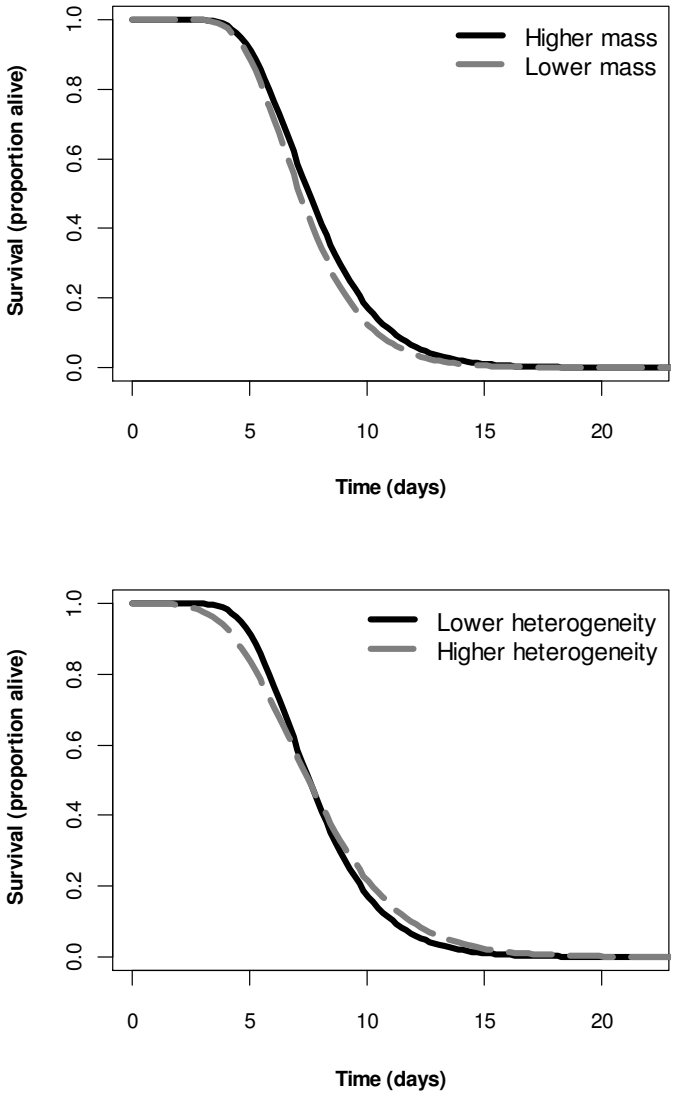

Figure 2.7. (A) Simulated survival curves of guppies from the two groups assuming they both have the same *s* and *u* parameters (those estimated for the M.NC group), but have different *r* parameters due to different masses at the onset of the challenge (higher mass group: *r* of M.NC group; lower mass group: *r* of M.C). (B) Simulated survival curves of guppies from two groups assuming they started with the same mass and consequently experienced the same average rate of vitality loss (*r* of NC.L group). The *s* and *u* parameters of the groups with lower and higher heterogeneity are those estimated for the NC.L and N.C groups, respectively. The *k* parameter was set to 0.

Discussion

With challenge experiments and vitality modeling, I examined whether intraspecific competition influenced variability among individuals and whether it had lasting effects through to cohort survival. The main result of this study was that food competition increased size variability among individuals, thereby increasing the number of individuals with high survival capacity. My experiments and simulations showed that a decrease in challenge survival could be eliminated with food competition. More specifically, the survival of the group with lower mass and no competition decreased on approximately day 10 of the challenge relative to the group with higher mass (Figure 2.7A); but in the group with an equally low mass raised under conditions with competition, survival was equivalent to that of the group with 20% more daily food rations without competition (Figure 2.4A). Intraspecific competition was observed as a force powerful enough to buffer against mortality. Higher cohort survival was possible due to the increased quality of highly competitive fish at the cost of less competitive fish. This may have significant impacts especially in species that have naturally occurring low survival to the reproductive adult stage such as fishes. Generally, 1%–7% of salmon smolts survive to the adult stage (Bradford 1995). More than doubling such a survival rate could have dramatic effects on cohort and population survival rates. Thus, my results suggest that foraging competition induced by temporally and spatially constrained prey resources is an important potential source of individual heterogeneity for increased population survival.

As an important basic finding prior to testing the effects of intraspecific competition on survival patterns, I verified the positive relationship between mass and survival. The survival patterns were indeed caused by the large differences in average body masses among treatment groups. The group with high food availability was four times greater in mass than the group with low food availability, and on average survived nearly seven times longer than the group with low

food availability. Most studies have found this relationship in fish between survival and size, mass, or growth (Sogard 1997, Persson and De Roos 2007, Jorgensen and Fiksen 2010), while some have not and hypothesized that the ranges they examined were not extensive enough (Letcher et al. 1996a). Also, initial vitality was strongly related to mass at the beginning of the challenge, suggesting that it is a good proxy of survival. The positive relationship between mass after the treatment stage and mass at time of mortality is similar to what Letcher et al. (1996a) observed, and is in agreement with other findings of mortality occurring at a critical threshold of energetic reserves (Biro et al. 2004, Crossin et al. 2004, Finstad et al. 2004, Rand et al. 2006). Overall, the large range of food availabilities tested is comparable to natural occurrences. Drastic differences in food availabilities can occur intra-annually between the growing season and the overwintering season when many fish starve (Shuter et al. 1980, Sogard and Olla 2000, Biro et al. 2004), or inter-annually in ocean conditions (Borja et al. 1998, Coronado and Hilborn 1998, Moss et al. 2005, Drinkwater 2006, Cross et al. 2009, Harwell et al. 2010).

When comparing less drastic differences in average body masses, variability among individuals had an important effect on the shape of the survival curves for fish in the four treatment groups. The competition-induced variability in mass among individuals caused greater survival than expected based on the initial average mass alone. This suggests that, given a set amount of resources, the greater the variability among individuals, the greater the benefits for late-surviving individuals. Growth variability has been identified in other studies as one of the most important factors of larval fish survival (Rice et al. 1993, Letcher et al. 1996b). The average growth rate of survivors may be greater than twice that of the initial mean growth rate (Rice et al. 1993). In this study, I found that in order to counteract the negative effects of 20% lower food density, nearly half of the initial group that experienced competition required greater relative vitality than the group not experiencing competition. My results suggest that increasing

heterogeneity among individuals could reduce population bottlenecks such as overwinter survival of young fish. Fish survival in the early life stages contribute considerably to year class strength or recruitment (Miller et al. 1988, Bradford and Cabana 1997, Cowan et al. 2000). Thus, this suggests that the sooner and the greater heterogeneity among individuals occurs, the greater are the benefits to population survival.

Vitality modeling (Li and Anderson 2009) is a convenient way to examine the subtle influences of individual heterogeneity as an underlying mechanism of cohort survival patterns. Over time, the combined effects of the two types of heterogeneities (initial and evolving) are important in quantifying the beneficial effects of overall heterogeneity on survival patterns. Initial heterogeneity can be thought of as the differences in survival capacities among individuals at birth or at the beginning of a life stage. In the present study, initial heterogeneity could be due to differences in genetic makeup and mass. Evolving heterogeneity, on the other hand, could be compared to growth depensation (i.e. increased size variability over time) (Pfister and Stevens 2002, Peacor et al. 2007) which is the increased variability among individuals over time. The benefits of initial heterogeneity are gradually overshadowed by evolving heterogeneity. As the distributions of relative vitality move toward the zero boundary, evolving heterogeneity causes the distributions to spread, thus resulting in gradually smaller proportions of individuals benefiting from initial heterogeneity. Thus, the greater the initial heterogeneity, the longer lived are the benefits to the individuals in the uppermost portion of the distribution of relative vitality.

Growth depensation can be affected by size-dependent and size-independent effects (Magnuson 1962, Pfister and Stevens 2002). Size-dependent effects are a result of positive feedback upon which individuals increasingly diverge in size due to, for example, their ability to capture resources and to avoid predation (Miller et al. 1988). In contrast, size-independent growth autocorrelation can be caused by uneven distributions of resources that consistently favor some

individuals, associative learning, genetic predisposition for social dominance, and trait variation in specialize foraging ability (Abbott et al. 1985, Abbott and Dill 1989, Hammer and Menzel 1995, Missoweit et al. 2007, Bell et al. 2009). Ontogenetic shifts can further increase variation among individuals and ties size-dependent and size-independent effects in a neat manner (Huss et al. 2007, Huss et al. 2008, 2010). Larger fish capable of feeding at the next trophic level transition from the planktivore to the piscivore stage. The higher energy-rich food consumed by piscivores increase their growth rate and fish size differences are thus further exacerbated. In contrast, if there are insufficient food resources at the lower trophic level, fish cannot grow large enough to feed on the next trophic level and variation among individuals can remain low. In my study, I cannot determine whether growth was related to size or to growth autocorrelation because each individual was not tracked through time, but both were possibly important. For the groups without competition, the initial pattern of masses at birth was likely overwhelmed by the satiating rations. The observed low CV of mass in these groups suggests that all individuals were able to grow equally well. While for the groups with competition, the initial pattern of masses at birth may have been exacerbated during the treatment stage. Larger or more dominant fish were able to capture more food and grow faster than smaller fish. Thus, size-independent factors were possibly more important for non-competing individuals, while size-dependent factors and genetically predisposed behaviors were both possible for competing individuals. Furthermore, food was localized at the front side of the aquarium which could favor competing individuals that were territorial, quicker at food handling, or better scramblers. But territoriality was likely not a significant phenomenon because dominant fish cannot efficiently defend territories at high population densities (Magnuson 1962). There were on average 27 individuals per 8-L tank in my study. Handling time which can contribute to the competitive abilities of dominant fish was also not likely a factor in my experiment because the size of brine shrimp nauplii were small relative

to the gape size guppies, even to those that were newly born. Their ability to perform in a "scramble" competition was most likely the case as food was only available for a limited amount of time. Scrambling abilities could be size-dependent or genetically predisposed specializations of physical traits or behaviors. Understanding the mechanisms of growth depensation would allow us to focus on particular processes that efficiently increase heterogeneity and survival in controlled settings such as hatcheries.

In fish aquaculture, practices are generally aimed at maximizing growth and food conversion efficiencies while minimizing effort (Storebakken and Austreng 1987, Giberson and Litvak 2003, Rowland et al. 2005). To reduce production costs, fast growth with minimum feeding frequencies and optimal rations, as well as high stocking densities are often employed. Although increased size variation among fish at high stocking densities has been documented (Jobling and Baardvik 1994, Irwin et al. 1999), how these growth patterns translate to survival is unknown. My results suggest that the increased size variation related to stocking densities can result in higher survival. For this reason, high stocking densities are beneficial, which contrasts the possible negative impacts such as increased pathogen transmission rates, greater aggressive behavior, and higher stress (Ashley 2007). Based on my results, mean body mass and heterogeneity among individuals are both important drivers of survival. A negative relationship appears to exist between increased growth and CV in growth (McCarthy et al. 1992, Xie et al. 2011). Thus, future research can investigate functional relationships between the mean and variability of growth rates – by controlling feeding frequencies, rations and stocking densities – and then determine their effects on survival.

In a larger context than what has been discussed thus far, the effects of domesticated populations (i.e. hatchery fish) on wild fish are of great concern to aquaculturists, resource managers, and conservationists. Hatchery fish are generally produced to supplement wild fish

stocks, but competitive interactions between hatchery and wild fish could result in displacement, lower fitness, and decreased population sizes of native stocks (McGinnity et al. 1997, Youngson and Verspoor 1998, McGinnity et al. 2003, Weber and Fausch 2003). To increase survival rates of wild stocks, my results can be interpreted as a need for hatchery fish to be poorer competitors than wild fish. However, the competitive ability that confers higher survival, as discussed earlier in relation to the current study, can be type-specific: interference (direct) competition and exploitative (indirect or scramble) competition (Ruzzante 1994, Ward et al. 2006). The former generally favors dominant fish that exhibit territoriality and aggressive behavior where food resources are spatially concentrated (Magnuson 1962). While the latter usually favors quicker reacting and faster swimming fish able to find and consume foods across spatial or temporal scales (Grant and Kramer 1992, Barber and Ruxton 1998). The successfulness of an individual in defending a territory or scrambling for a food resource also depends on population density (Magnuson 1962, Magellan et al. 2011). As the number of subordinate fish increases, the dominant fish is less successful at securing food. Thus, relating competitive abilities to survival depends on the ecological context. Determining the mechanisms underlying the competitive interactions would help establish the effects of heterogeneity on survival. Furthermore, hatchery Atlantic salmon haven been regarded as biologically different from their wild counterparts, and could be considered a new species (Gross 1998). The competitively and reproductively inferior hatchery fish likely decrease the fitness of wild fish (McGinnity et al. 1997, Fleming et al. 2000, McGinnity et al. 2003, Hutchings and Fraser 2008). Thus, our results in this context would suggest that inferior hatchery fish are useful in buffering against the mortality of wild fish, but there is also the need for reproductive isolation between the two types.

In conclusion, my findings suggest that increased inequality in foraging success among individuals can further benefit the individuals with high vitality. Although the low vitality

individuals may suffer from increased mortality at early life stages, the increased survival of high vitality individuals at the reproductive stage is most important. This "buffering effect" on survival can occur within life stages, and the cumulative effects across life stages would increase cohort and population survival. The degree of effect will depend on the underlying mechanisms which may be size-dependent or size-independent. Behaviors related to fish population densities and the spatial and temporal distributions of food are also important. Identifying these mechanisms will be important when determining the effects of intra-specific and possibly inter-specific competition in a target population of hatchery fish or both hatchery and wild fish. This study is the first to demonstrate the effects of competition on variability in body masses and subsequently cohort survival, and facilitates future studies on the functional relationships involving drivers of growth depensation and population survival.

Chapter 3

Step-like Pattern in Survival Reflects Selection Processes

Abstract

Multiple selection processes can occur across and within life stages, but are not often depicted in survival curves. In this study, I challenged juvenile hatchery rainbow trout (*Oncorhynchus mykiss*) with two stressors (increased water temperature and absence of food resources) known to occur across different time scales. The time to mortality of the challenged rainbow trout revealed a step-like pattern resembling a two-humped survival curve. The first step, which occurred in the first 20 days of the challenge experiment, was assumed to occur from thermal stress. The second humped curve, which occurred from approximately challenge day 20 to 62, represented fish capable of acclimating to the increased water temperature, but died from starvation. Rainbow trout that died from starvation lost on average 58% of the energy density of control fish sampled at the onset of the challenge experiment. The same challenge experiment conducted on juvenile hatchery Chinook salmon (*O. tshawytscha*) sampled in the Columbia River revealed that step-like patterns in survival can also be elicited in field collected samples. However, the selection processes likely involved different aspects of thermal stress and not a depletion of energetic reserves because survival was less than 10 days in all tested Chinook salmon. In addition to the investigation of step-survival patterns, the loss of equilibrium (LOE) surrogate endpoint was examined for potential replacement of spontaneous mortality in challenge experiments and consequent reduction of the sense of pain in fishes. A group of rainbow trout in the challenge experiments were observed for LOE (LOE group) and compared to those that spontaneously died (Mortality group). The difference in time to LOE and time to mortality (Δt) increased as survival of the Mortality group (l_M) decreased from 1 to 0.5, and then decreased as l_M

continued to drop from 0.5 to 0. This non-constant pattern Δt reveals the importance of heterogeneity among individuals and the underlying biological processes of LOE and mortality.

Introduction

Organisms with several life stages can experience a number of bottlenecks to survival throughout their lifetime (Cunjak et al. 1998, Armstrong et al. 2003). One example is the limited availability of shelters, juvenile habitat, covered migratory corridors, and adult habitat for crabs and lobsters at risk of size-selective predation (Beck 1995, 1997, Butler and Herrnkind 1997, Halpern 2004). Also, abrupt decreases in survival probabilities at the beginning of each life stage have been observed in birds (Low and Pärt 2009). Even within a life stage, an organism can face more than one selection process (Hurst 2007). For many freshwater fishes, the first overwinter survival is a bottleneck due to limited food resources, size-selective predation, extreme temperatures, and pathogens (Ludsin and DeVries 1997, Biro et al. 2004, Hurst 2007). Although episodes of natural selection and lifetime (or life stage) survival curves are two fundamental topics in ecological research, studies on the fusion of the two in the form of step-survival curves is infrequent (Yashin et al. 2002, Wu et al. 2006). Conceptually, at every episode of natural selection, a drop in survival would occur followed by a step. Figure 3.1 provides an example of a lifetime survival curve of an anadromous fish with a few step-like patterns. The number of humps in the survival curve would represent the number of episodes of natural selection across life stages.

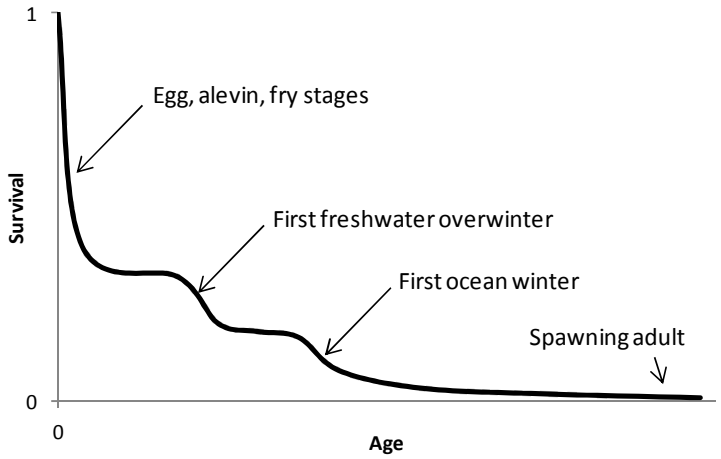

Figure 3.1. Hypothetical example of a step-survival curve depicting episodes of natural selection throughout the lifetime of an anadromous fish.

The study of relative survival within a life stage can be conducted by challenge studies of individuals subjected to stressors such as increased water temperature, absence of food resources, predation, pathogens, and toxins (Coutant 1973, Paladino et al. 1980, Anderson 2000, Arkoosh et al. 2006). Increased water temperature (or thermal stressor) has often been used in challenge studies because of its relative ease of control and its use in determining a fish's degree of tolerance as an indicator of past conditions experienced (Brett 1952, Hutchinson 1976, Wedemeyer et al. 1990). Mortality from thermal stress is relatively quick and occurs over a time scale of days (Brett 1952, Paladino et al. 1980), relative to mortality from starvation which occurs after weeks or months (Biro et al. 2004). Increased water temperature and the absence of food resources would make ideal stressors in a challenge study to reproduce a step-survival curve in a fish species.

Loss of equilibrium (LOE) has been used as a surrogate endpoint to replace spontaneous mortality to minimize nociception experienced by test animals. It also has been utilized because the exact time of mortality can be difficult to pinpoint visually (Wedemeyer et al. 1990). LOE is generally considered a reasonable indicator of mortality because fish that exhibit this behavior

soon die unless they are transferred to more optimal water conditions, and even then few recover. LOE is a characteristic response of fish to some stressors such as prolonged exposure to increased water temperature (Dean 1973, Van Dijk et al. 1999). As fish experience thermal stress, the tail first drops, a phase at which they can still regain equilibrium, and then roll over (i.e. lose equilibrium) and cannot regain an upright position (Dean 1973). LOE has been used as a surrogate endpoint in many studies of critical thermal maxima (Cox 1974, Friedlander et al. 1976, Paladino et al. 1980), but only averages or medians were determined. Because LOE is a common response during thermal stress that precedes mortality, it is a potentially useful surrogate endpoint to test as a predictor of time to mortality during challenge experiments.

The two major objectives of this study were to 1) examine the effects of two stressors hypothesized to induce mortality at different time scales and consequently result in a step-survival curve, and 2) determine how time to LOE can be used as a predictor of time to mortality. These two objectives were accomplished in a challenge experiment with juvenile rainbow trout (*Oncorhynchus mykiss*) transferred directly from a hatchery to research facilities. Additionally, the first objective was also carried out with hatchery yearling Chinook salmon (*O. tshawytscha*) sampled from the Columbia River (Washington) to reveal whether step-selection curves can be elicited from field-collected samples.

Methods

The effects of stressors on the shape of a survival curve were examined in two sets of experiments. The first set of challenge experiments, that included two stressors of increased water temperature (24°C) and the absence of food resources, was tested in hatchery juvenile rainbow trout (*Oncorhynchus mykiss*) from 13 December 2007 to 16 February 2008. The experiments

conducted with rainbow trout were designed to determine the pattern of survival (*l*) throughout the challenge experiment, to verify that starvation was indeed one of the two stressors tested, and as a separate research investigation, to test loss of equilibrium (LOE) as a surrogate endpoint to spontaneous mortality. The second set involved a challenge experiment with a thermal stressor (24°C) without food (but not necessarily resulting in starvation) conducted with juvenile hatchery spring/summer Chinook salmon (*Oncorhynchus tshawytscha*) collected in the field from 21 April to 2 June 2008. These experiments were performed under protocol #3382–03 approved by the Institute of Animal Care and Use Committee at the University of Washington.

For the first set of experiments, the juvenile rainbow trout were trucked directly from Nisqually Trout Farm (Olympia, Washington) to an aquatic laboratory facility at the University of Washington. The two hundred rainbow trout tested were bulk weighed in groups of four to nine fish and averaged 6.8 g in wet mass. The rainbow trout were then placed into a tank system with water re-circulating in parallel at a density of 25 individuals per 114-liter tank. They were acclimated for 1.5 days at 9.5°C with an aquarium chiller. Please see Appendix B.1 and B.2 for additional information on water quality, lighting, temperature conditions, and fish densities. At the onset of the challenge (i.e. once 24°C was reached), a random subsample of rainbow trout (Control group; n = 20) was euthanized with tricaine methanesulfonate (MS-222; 250 mg/L buffered to 7.0 with $NaHCO_3$). Among the remaining rainbow trout, 90 individuals housed in four tanks were designated as the Mortality group and were sampled only at time of spontaneous mortality. The other 90 individuals housed in another four tanks were designated the LOE group and were sampled when they lost equilibrium and could not regain an upright position. A number of rainbow trout in this group spontaneously died before any signs of LOE were detected, and were sampled at their time of mortality. The rainbow trout were observed for mortality and LOE at least 3 times a day at approximately 09:00, 16:00, and 22:00. If any rainbow trout showed signs

of health issues, they were euthanized and removed from experiment. This only occurred to one rainbow trout in the Mortality group. One survival curve was fit to the observed time to mortality and one for the time to LOE with a vitality model (Li and Anderson 2009). For a description of the vitality model, please see the Methods of Chapter 2 (Resource Competition Induces Heterogeneity and Beneficial Population Selection).

The rainbow trout were hypothesized to have died from energy depletion if their energy density reached a critical threshold, and assumed to have died from thermal stress if their energy density did not reach this critical threshold. After sampling, the fish from the Control, Mortality, and LOE groups were processed for wet mass, dry mass and a subset of each group for energy density. The bomb calorimeter (SemiMicro Bomb Calorimeter 1425, Parr Instrument Company®) was calibrated with "Tris calibration standard" prior to processing samples. In the order of when the rainbow trout were sampled from the challenge experiment (i.e. challenge day), approximately every other fish was processed for energy density. The sample sizes were 12 rainbow trout in the Control group, 42 in the Mortality group, and 46 in the LOE group. To measure the precision of processing by bomb calorimetry, replicates were run on 5 samples. The standardized difference was calculated as:

$$\text{standardized difference} = \frac{|\, replicate_1 - replicate_2 \,|}{replicate_1} \times 100\%$$

The standardized difference was 0.9% ± SE 0.2%. A linear regression between energy density and challenge day was determined for an "early" period in which rainbow trout from the Mortality group were assumed to die from thermal stress, and a "late" period in which they were assumed to have died from starvation stress.

In a second set of challenge experiments, the field-collected samples were of hatchery yearling Chinook salmon (mean individual mass of 30.0 g; no SD because weighed in bulk) that

experienced two types of passage (run-of-river, ROR; barged, B) through the Federal Columbia River Power System (Oregon and Washington). For further information on the methods of field sampling and laboratory experimentation, please see Methods of Chapter 4 (Survival Capacity of Chinook Salmon After Two Types of Passage Through a Hydropower System in the Snake and Columbia Rivers). Unlike the rainbow trout challenge experiments, the time to mortality and time to LOE of the Chinook salmon were used together to produce one survival curve for each field sample. The time between LOE and mortality often occurred in over a time scale of minutes and thus Δt was assumed negligible. Survival curves were again fit to the observed time to mortality/LOE with a vitality model (Bell et al. 2009). The purpose of the second set of challenge experiments in the context of this study was to determine whether step-survival curves occurred in fish collected in the field. For a comparison of survival capacity of these fish with different types of passage through the hydropower system, please see Chapter 4.

In the investigation of LOE as a surrogate endpoint, linear regressions were determined between the difference in time to mortality and LOE (Δt) and survival of rainbow trout in the Mortality group (l_M). Because 90 fish were tested in each group, the survival for each fish could be matched between the two groups, and Δt could be directly calculated. Only fish from the LOE group for which LOE was detected were included in this analysis, and the one fish in the Mortality group that was euthanized for health issues was excluded in this analysis. Thus, the sample size was reduced to 65 paired individuals for the determination of Δt. Two linear regressions were fit to the data of Δt, one for the survival of the Mortality group that was $1 < l_M < 0.5$, and one that was $0.5 < l_M < 0$. The assumption of linearity was not met for first linear regression of Δt and transformations did not help alleviate non-linearity. The results were thus interpreted with caution, and a subset of the data was also fit with a logistic regression. Energy

densities by J/g wet weight of the Control, LOE, and Mortality groups were compared to help explain patterns observed in Δt.

Results

The juvenile rainbow trout introduced to a challenge experiment of thermal and starvation stress exhibited a step-like pattern in their survival (i.e. l_M, proportion alive for the Mortality group; l_{LOE}, proportion with equilibrium for the LOE group) which the fitted survival curve did not capture (Figure 3.2). The observed survival pattern before challenge day 20 exhibited one humped curve, that was followed by another humped curve that extended to the end of the challenge experiment (challenge day 62). Two distinct patterns were also observed in the energy density of the rainbow trout in the Mortality group (i.e. measured at time of mortality; Figure 3.3). Energy density significantly declined as rainbow trout died between challenge days 0 and 20 (linear regression: $y_A = 5492 - 133.7x_A$, $r^2 = 0.451$, $p = 0.017$). Rainbow trout that died after challenge day 20 reached a constant critical threshold of energy density (linear regression: $y_B = 2149$, $r^2 < 0.001$, $p < 0.001$). The mean energy density was highest at the onset of the challenge, lower between challenge days 0 and 20, and lowest after challenge day 20 (Figure 3.3). Overall, across a similar temporal scale, the patterns of survival and energy density occurred in the challenge experiment through two different processes: 1) initial mortalities (i.e. before day 20) were presumably associated with thermal stress rather than energetic depletions because the fish still contained relatively high energy densities, and 2) later mortalities (i.e. after day 20) were related to starvation because the fish reached a low critical threshold.

Juvenile Chinook salmon with two different types of passage through the Federal Columbia River Power System often exhibited a step-like pattern in their survival curves during

the challenge experiments (Figure 3.4). The maximum duration of the challenge trials spanned between 1.3 and 9.5 days, in contrast to the juvenile rainbow trout study that lasted 62 days. Li and Anderson's (2009) vitality model also could not capture the step-like pattern of these survival curves. For the ROR2.08 sample, the model failed to converge to a solution.

The time to the surrogate endpoint LOE almost always occurred before the time to mortality in the challenge experiment conducted with juvenile rainbow trout (Figure 3.2). The difference in these two times (Δt) resembled the shape of a candle flame (Figure 3.2). At a given survival, Δt significantly increased between $1 > l_M > 0.5$ (linear regression: $\Delta t = 31.0 - 34.3l$; $r^2 = 0.836$; $p < 0.001$; Figure 3.5), and then significantly decreased between $0.5 > l_M > 0$ (linear regression: $\Delta t = 1.66 + 18.7l$; $r^2 = 0.754$; $p < 0.001$; Figure 3.5). The logistic regression fitted to the data from approximately the first half of the challenge experiment visually appears to provide a better fit than the linear regression.

Rainbow trout in the LOE group had greater energy density than those from the Mortality group within the "early" (before day 20) and within the "late" (after day 20) periods of the challenge (Figure 3.6A). However, among the individuals in the LOE group, the energy density of the fish during the challenge period of 0 to 20 days was not significantly different between individuals for which LOE was and was not observed before they died (t-test: $t = 0.422$, $df = 8.46$, p-value = 0.683; Figure 3.6B). After challenge day 20, the energy density was significantly higher in rainbow trout for which LOE was observed than in those for which LOE was not observed before they died (t-test: $t = -3.66$, $df = 19.9$, p-value = 0.002; Figure 3.6B).

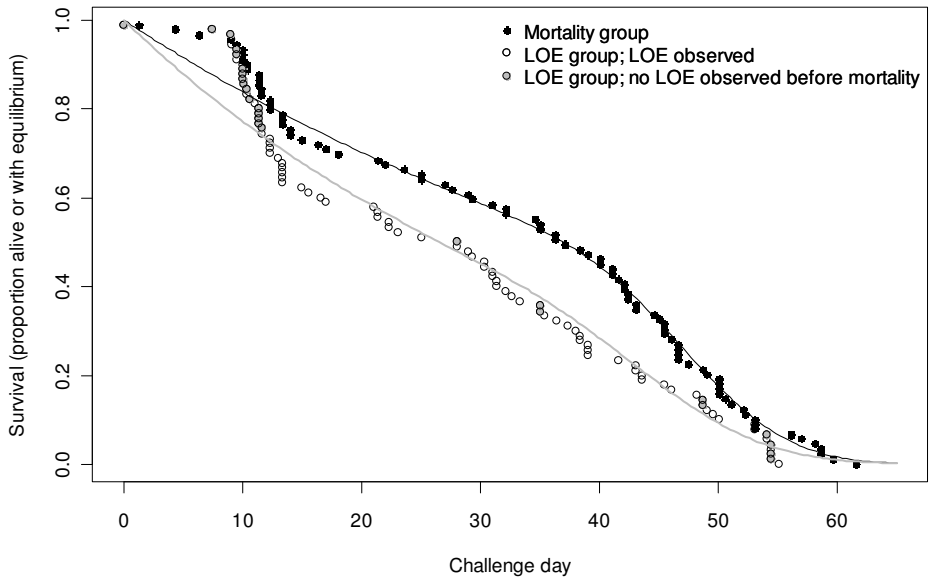

Figure 3.2. Observed (points) and fitted (lines) survival (l) of rainbow trout during a challenge experiment with two stressors (increased water temperature and absence of food resources) based on spontaneous mortality or loss of equilibrium (LOE). Survival curve fitted by Li and Anderson's (2009) vitality model.

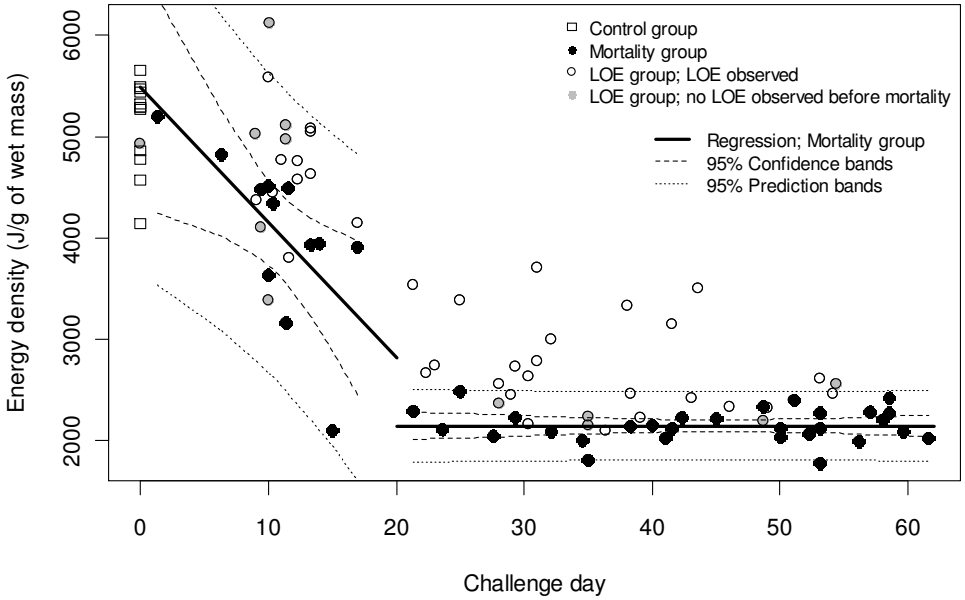

Figure 3.3. Energy density of rainbow trout introduced to a challenge with two stressors (increased water temperature and absence of food resources). The decrease in energy density of rainbow trout in the Mortality group (fish that spontaneously died) prior to challenge day 20 is represented by one linear regression ($y_A = 5492 - 133.7x_A$, $r^2 = 0.451$, $p = 0.017$), and the critical threshold of energy density after challenge day 20 is represented by another linear regression ($y_B = 2149$, $r^2 < 0.001$, $p < 0.001$). Energy densities of rainbow trout from the Control and LOE groups are plotted for qualitative comparison to those from the Mortality group.

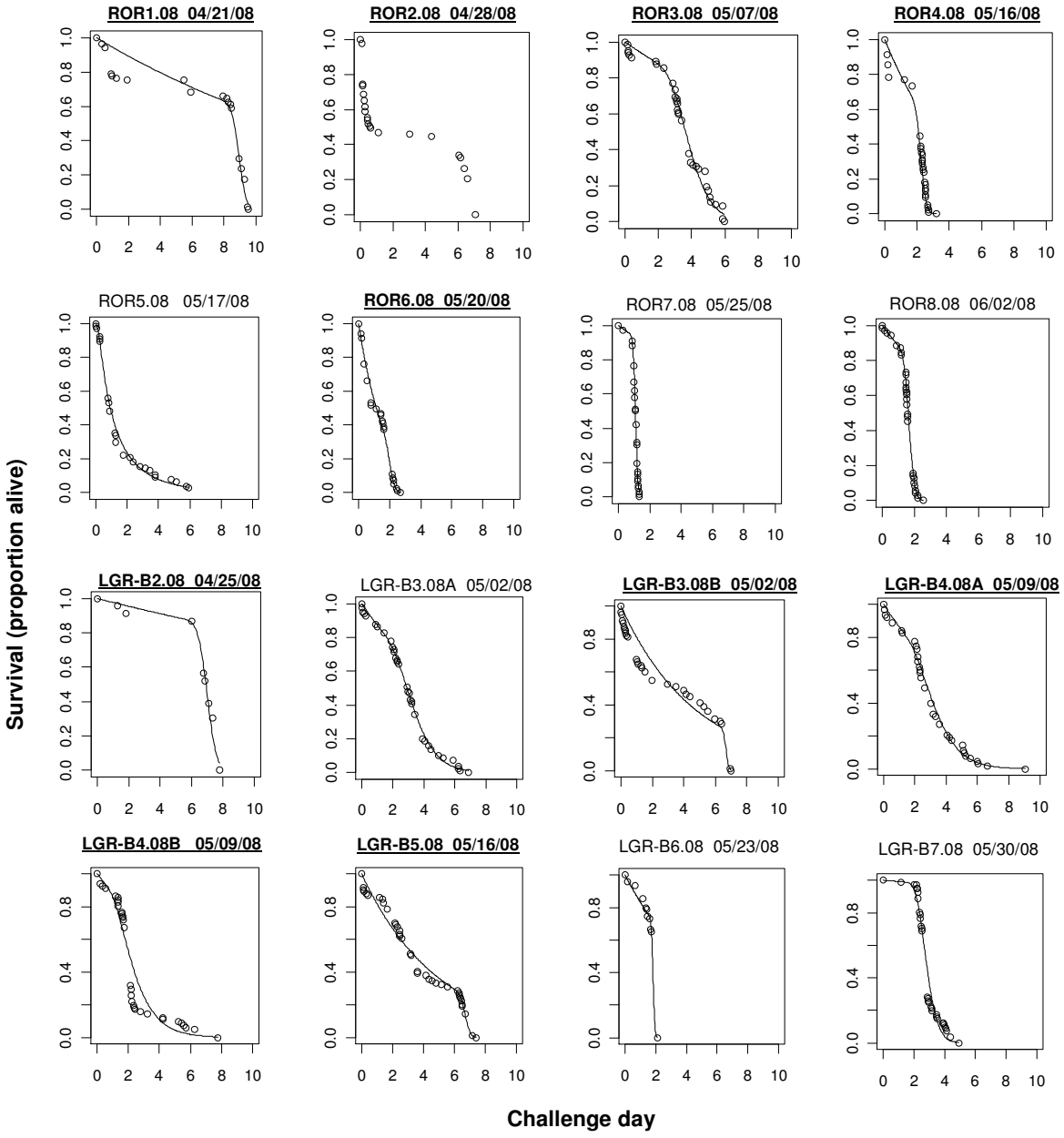

Figure 3.4. Challenge experiment of increased water temperature without food: observed survival (points) and fitted survival curves (lines) by the Li and Anderson (2009) vitality model of juvenile spring/summer Chinook salmon with different types of passage through a hydropower system (run-of-river, ROR; barged from Lower Granite Dam, LGR-B). Dates above graphs denote time of collection at Bonneville Dam. Sample sizes for each treatment group are the "N (challenged)" in Table 4.1. Survival curves that visually appear to exhibit step-like patterns are represented by bolded and underlined sample names and dates.

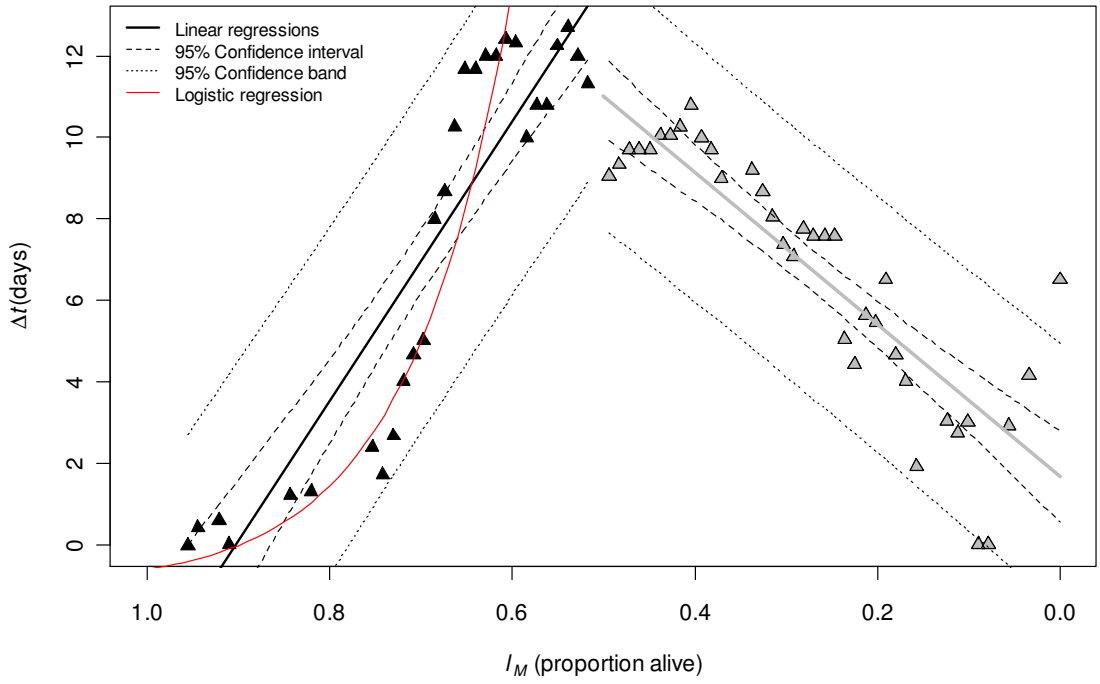

Figure 3.5. Relationship between the difference in time to mortality and time to LOE (Δt) by the Mortality group survival (l_M). Black triangles and black line respectively represent the observed data and linear regression ($\Delta t = 31.0 - 34.3l$; $r^2 = 0.836$; $p < 0.001$) determined for $1 > l_M > 0.5$, while the grey triangles and grey line respectively represent the observed data and linear regression ($\Delta t = 1.66 + 18.7l$; $r^2 = 0.754$; $p < 0.001$) determined for $0.5 > l_M > 0$. The red line represents the logistic regression ($\Delta t = \frac{3132}{1+e^{8.9l_M}} - 1$) for $1 > l_M > 0.6$. Please note that the x-axis decreases from 1 to 0.

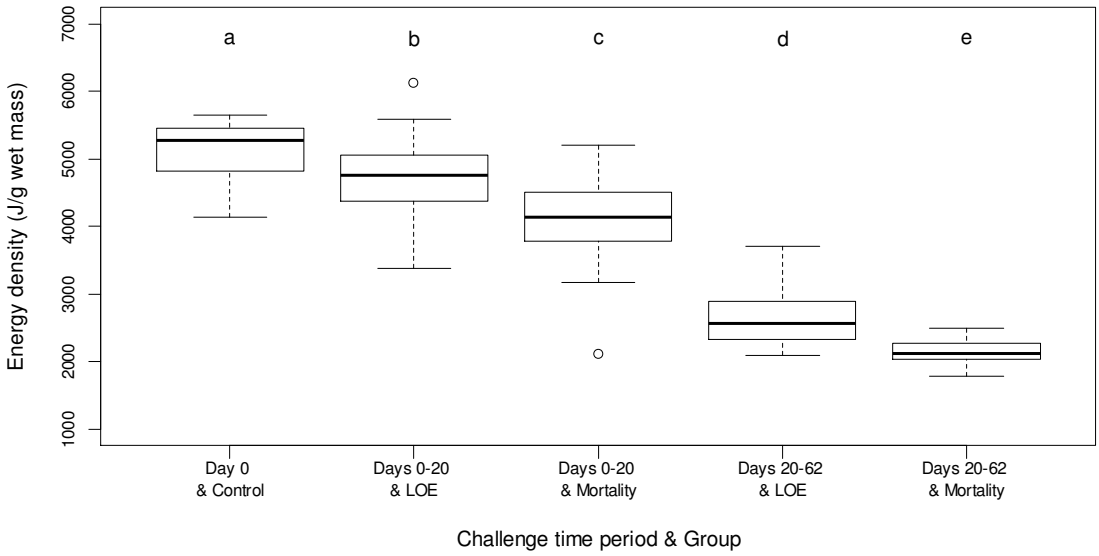

Figure 3.6A. Median (thick line), first and third quartiles (box), and 1.5 times the first and third quartiles (whiskers) of energy density for the Control group, and the LOE and Mortality groups before and after challenge day 20. Data points represent outliers that are beyond 1.5 times the first and third quartiles. The different letters at the top of the boxplot represent statistical differences between groups at $\alpha = 0.05$.

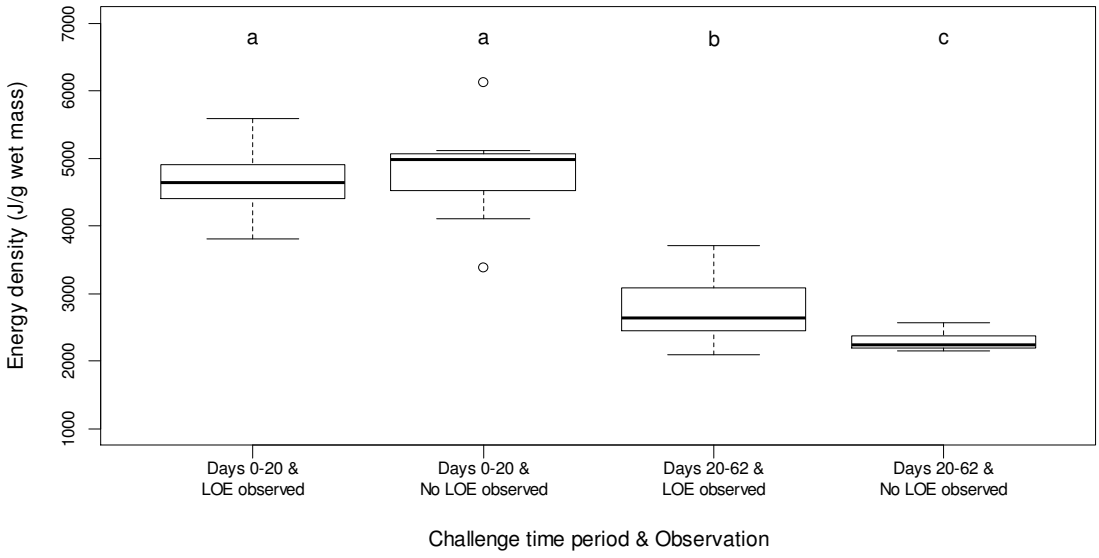

Figure 3.6B. Median (thick line), first and third quartiles (box), and 1.5 times the first and third quartiles (whiskers) of energy density for the LOE group, with or without LOE observed, during the two major time periods of the challenge. Data points represent outliers that are beyond 1.5 times the first and third quartiles. The different letters at the top of the boxplot represent statistical differences between groups at $\alpha = 0.05$.

Discussion

Selection processes

The survival pattern and energy density data of the rainbow trout challenge experiments suggest that two selection processes were occurring at different time scales: one that occurred in less than 20 days by a short-term thermal stressor, and one that occurred after 20 days by a long-term starvation stressor. Individuals that acclimated to the short-term thermal stressor, eventually died from longer-term starvation stressor. The vitality model (Li and Anderson 2009) could not capture the two-humped survival pattern because it was designed for one selection process, or an averaging of multiple selection processes, that results in a one-humped survival curve. A two-selection process version of the vitality model has been developed and provides a better fit to the data of times to mortality than the one-selection process version (Appendix B, Figure A.2; Li et al. *In preparation*).

Out of the two selection processes, there is strong support for starvation as the long-term stress experienced. The energy density data showed that the rainbow trout on average reached their critical threshold when they dropped 58% in energy density. This rate is similar to those observed in other studies (Biro et al. 2004, Crossin et al. 2004, Rand et al. 2006). The starvation experiments of Biro et al. (2004) conducted in the laboratory suggested that there was a critical threshold for lipid reserves at approximately 0.001 g lipids·g^{-1} wet mass. Those with the lowest lipid reserves died at about 30 days, and those with the highest lipid reserves died at about 80 days. This is comparable to the time of starvation that spanned 20 to 62 days at higher water temperatures in this study. Biro et al. (2004) also observed the starvation of age-0 rainbow trout in an experimental lake experiment. Rainbow trout that died had just under 60% loss of lipid reserves for their large-sized class (average mass of 0.75 g) and just over 90% loss of lipid reserves for their small-sized class (average mass of 0.41 g). Again these rates are comparable to

the 58% loss in energy density of my rainbow trout that originally weighed 6.8 g on average. In comparison to migrating adult sockeye salmon, their critical threshold was 4 $KJ \cdot g^{-1}$ (Crossin et al. 2004, Rand et al. 2006) which is higher than the critical threshold (2 $KJ \cdot g^{-1}$) determined in the juvenile salmonid I tested. The critical threshold determined in immature (2-year and 3-year old) Atlantic salmon (*Salmo salar*) was also close to this range at 4.4–4.8 $KJ \cdot g^{-1}$ (Finstad et al. 2004). The range of energy densities has been observed to reach below 3 $KJ \cdot g^{-1}$ (Rodgveller et al. 2007). Thus, my data suggests that critical thresholds of juvenile salmon, particularly in controlled laboratory settings, are lower than for adults in field settings. Overall, starvation was likely the long-term stress experienced by rainbow trout in my study.

The short-term stressor was assumed to be a thermal stressor. The mechanisms by which fish die at critically high temperatures include a variety of processes. Mortality from thermal stress has been thought of as a systemic breakdown (Fry 1958), that can occur within the anatomic and central nervous systems (Brett 1956, Baldwin and Hochachka 1970, Van Dijk et al. 1999), in the cell membrane (Bowler and Manning 1994), and at the subcellular level (Van Dijk et al. 1999). Increased variability in ionic and water balance in teleosts experiencing thermal shock suggests a destabilization of regulatory systems (Houston 1973). Temperature acclimation appears to stem from the central nervous system (Fry and Hochachka 1970). Experiments with eel in a swim chamber that allowed two different temperatures to be experienced by the anterior and posterior halves of their bodies suggest that the central nervous system was the regulatory control site (Precht 1961, Schultze 1965ab, from Fry and Hochachka 1970). This suggests that a breakdown in the central nervous system at the site of the brain occurs before a breakdown of cells in the rest of the body due to this loss of regulatory control. Another major driver of mortality from thermal stress appears to be the oxygen deficiency due to the inactivation of the respiratory nervous system (Brett 1956). The thermal window of performance (that can include

the biochemical and aerobic capacity of cells and tissues, and the capacity of organs to supply oxygen beyond that of maintenance processes) matches the window of aerobic scope (Pörtner 2010). Anaerobiosis induced by high temperatures is an early indicator of a critical threshold in temperature (Zielinski and Pörtner 1996). The anaerobic end products, lactate and succinate, increased and then returned to control levels within 3 days in the heart and muscle, but continued to increase after 1 day in the liver (Van Dijk et al. 1999). The authors suggest that an insufficient supply of oxygen to organs, particularly the liver, may be caused by impaired respiration, poor blood circulation, or both. They also suggest that an accumulation of anaerobic end products could lead to death. Pörtner and Knust (2007) have found that the deficiencies in oxygen delivery, that were caused by increased water temperatures, matched the environmental temperatures at which there are decreases in population abundance and individual growth. At a broader level of biology, mortality from thermal stress can be caused by a breakdown of certain critical cells (Fry 1958). There is a conflict at the cellular level to maintain regular activities and to produce the ability to resist extreme temperatures (Christophersen and Precht, Fry 1958). The biophysical and biochemical restructuring of cells, particularly the synthesis of new proteins in the lipid bilayer of cell membranes, help to maintain metabolic control and to help stabilize the rates of reactions catalyzed by enzymes during acclimation (Fry and Hochachka 1970). The difference among individuals may be due to the unique composition and architecture of the cell membrane and in the subcellular space, a concept similar to how isozymes are linked to the degree of acclimation. Overall, mortality from thermal stress is likely to occur due to deficiencies in oxygen delivery for maintenance and performance of the fish, a loss in the precision of biochemical regulation and an overall system destabilization.

In my study, there may have been two major phenotypes: one that was not able to acclimate to the thermal stressor and died before starvation occurred, and another that acclimated

to the thermal stressor but then died from starvation. The distinction between these two major phenotypes is likely linked to genetic effects and environmental conditions. Gibson (1954) observed step-like patterns of survival in guppies (*Poecilia reticulata*) challenged at 34°C that were no longer exhibited after the third generation of inbreeding and in select crosses between siblings. The environmental conditions also play a key role in bringing out these two phenotypes. At temperatures close to the upper incipient lethal temperature (UILT), in which indefinite exposure causes 50% mortality of the individuals challenged (Fry et al. 1946), step-like patterns in survival can be elicited (Brett 1952, Gibson 1954). However, at temperatures greater than the UILT, these step-like patterns in survival disappear; and at temperatures lower than the UILT, fish can resist the relatively warm water temperatures and survive long-term. Also, a gradual increase in water temperature can allow partial acclimation to occur (Cox 1974). These step-like patterns in survival, likely caused by varying degrees of acclimation controlled by genetic and environmental influences, were reproduced in my study with the addition of starvation identified as the long-term stressor.

One possible way, related to genetic differences and previously experienced conditions, that the rainbow trout acclimated to the conditions in my challenge experiments was by producing 70 kDa heat shock proteins (hsp70) which are known to maintain proteins by repairing the denatured ones or translocating the permanently damaged ones to lysosomes and proteasomes for breakdown and removal (Kiang and Tsokos 1998, Basu et al. 2002, Lund et al. 2002). Hsp70 was first discovered in studies conducted with *Drosophila* exposed to thermal stress (Basu et al. 2002). These hsps are also known as stress proteins because of their up-regulation in response to many stressors other than heat, particularly those instigating the denaturation of proteins. Concentrations of hsp70 in fish tissues can be good measure of stress, or protection against stress (Parsell and Lindquist 1993, Iwama et al. 1998, Podrabsky and Somero 2004). A link between an

organism's temperature tolerance and the temperature limit of hsp70 has been determined in several studies (White et al. 1994, Norris et al. 1995, Hightower et al. 1999). Also, differences in the magnitude of induction among isoforms of hsps, including *hsc70*, *hsp70-1* and *hsp70-2* as well as *hsp90*α and *hsp90*β may explain differences in temperature tolerances among populations (Fangue et al. 2006). The levels at which isozymes are activated within a species can vary more widely than between species that have been adapted to different thermal environments (Fry and Hochachka 1970). Hsps are one of many genetically and phenotypically-linked proteins that could affect their abilities to acclimate to thermal conditions. Antioxidants also can affect thermal tolerance of organisms (Pörtner 2001); differences in the types and quantities of these can influence whether an individual is capable of acclimating to the stressful conditions or whether it dies.

The survival patterns of juvenile Chinook salmon in the field suggest that a two-selection process, or even multiple-selection process, can occur naturally. Although, the two selection processes of these Chinook salmon were likely both not the same as the two in the rainbow trout study. Starvation occurs over a matter of weeks or months, and not days. Thus, starvation was likely not the second stress experienced by the Chinook salmon. The relatively short time scale of the challenge trials of Chinook salmon suggest that the stressors involved a disruption of physiological and energetic processes linked to deficient oxygen delivery. Defense mechanisms regulated by the central nervous system and heat shock proteins, for instance, were possibly not capable of stabilizing systems at the anatomic, cellular, and subcellular levels. The pattern in the survival curve is thus dependent on the fish's condition and the selection processes in the environment. Step-survival patterns are indicative of phenotypic variation among individuals and selection processes occurring at different time scales.

Loss of equilibrium

To the best of my knowledge, this study is the first to examine the pattern in the time difference between loss of equilibrium and mortality (Δt) throughout a whole survival curve. Loss of equilibrium, similar to mortality from thermal stress, is thought to be caused by a systemic disorganization and failure of the central nervous system (Cox 1974, Friedlander et al. 1976, Van Dijk et al. 1999). As my challenge experiment progressed, the increase and subsequent decrease in Δt suggest that: 1) early in the challenge individuals were highly stressed and quickly died after losing equilibrium; 2) towards the middle of the challenge (i.e. when survival l was approximately 0.5), individuals may have lost equilibrium but the mechanisms that caused LOE were not sufficiently severe enough to cause immediate mortality; and 3) towards the end of the challenge, once the central nervous system and other regulatory processes were destabilized, LOE occurred and mortality shortly thereafter ensued. How this pattern in Δt arose depends on the mechanisms of LOE and mortality from thermal stress, both of which are still being examined after decades of research (Brett 1956, Fry 1958, Fry and Hochachka 1970, Cox 1974, Friedlander et al. 1976, Van Dijk et al. 1999). Different biological processes including depletion of energetic reserves are probably occurring in fish to result in either LOE or spontaneous mortality. This is supported by 1) the significant differences in energy density I observed between the LOE and Mortality groups early and late in the challenge experiments, 2) the lack of significant differences between those that did and did not lose equilibrium before mortality in the LOE group early in the challenge, and 3) the significant differences between those that did and did not lose equilibrium in the LOE group late in the challenge experiment. On the whole, the discovery of a non-constant Δt, and even more so, a predictable pattern of Δt, reinforces the importance of considering heterogeneity among individuals.

Although significant linear relationships were determined between Δt and l_M, a non-linear relationship may have been more appropriate, especially for when $1 > l_M > 0.6$. This may have been related to the thermal stress that the some of the rainbow trout experienced early in the challenge experiment. It is important to note that no replicates were conducted, and whether these patterns are consistent across replicates, remains to be tested. Thus, it is unknown whether a simple linear model is sufficient, and perhaps may be more accurate on average across replicates than a non-linear model.

Applying the surrogate endpoint of LOE as the true endpoint may be more ecologically realistic because once a fish loses equilibrium, it is "ecologically" dead (Coutant 1969, Dean 1973). Sublethal thermal stress can increase a fish's susceptibility to predation (Coutant 1973). Ecologically, the rainbow trout that survived the longest in my study may be considered the fittest among all individuals tested for two reasons. They endured starvation the longest, and also exhibited LOE the latest, a behavior which would have likely resulted in predation. In contrast, the fish that died when survival l was approximately 0.5, were most disproportionately disadvantaged because of their relatively early onset of LOE and possible increased predation risk. Even without predation, latent mortality has been observed to occur 12 hours after fish have regained equilibrium (Dean 1973). More specifically, among the Chinook salmon that were acclimated at 15°C, exposed to 27°C–30°C, and then returned to 15°C at the moment LOE occurred, 80% of the Chinook salmon that exhibited LOE, did not recover. This suggests that sublethal exposures can be cumulative and consequently induce mortality. Overall, LOE can be a good indicator of a fish's condition in an ecological context because of its involvement in increased predation risk and cumulative stress.

Overall, organisms likely face multiple selection processes, and heterogeneity among individuals will result in different survival patterns for populations. The results of my challenge experiments conducted on rainbow trout (transferred directly from a hatchery) and Chinook salmon (collected in the field) suggest that a step-survival curve can result from two selection processes occurring at different time scales and two phenotypic groups related to acclimation abilities. This study supports continued use and development of analyses that incorporate multiple selection processes at various life stages such as life cycle models. Loss of equilibrium, which also showed a step-like pattern in its timing, may be a more ecologically appropriate endpoint. But if used as a surrogate endpoint to mortality, the non-constant pattern (but rather a "candle flame"–shaped pattern) of Δt needs to be considered due to the timing of the different underlying mechanisms of LOE and mortality across individuals. Future studies can examine the underlying mechanisms of thermal stress, and how previous experiences can alter the timing of these endpoints. This study further supports the hypothesis of reduced tolerance to increased temperatures due to previously experienced stress, and emphasizes the importance of heterogeneity among individuals.

Chapter 4

Survival Capacity of Juvenile Chinook Salmon After Barge and Run-of-River (ROR) Passage Through a Hydropower System

Abstract

Experiences from one life stage can affect survival capacity in subsequent life stages of individual organisms. Understanding these cross-life stage relationships in environments heavily modified by humans will help in determining the effectiveness of conservation strategies for populations. In the Federal Columbia River Hydropower System (FCRPS), anadromous Pacific salmon (*Oncorhynchus* spp.) can pass between and through the dams by different routes: 1) run-of-river (ROR) migration, which results in relatively high direct mortality rates, and 2) barge transportation, a mitigation strategy that eliminates nearly all direct mortality. Differences in indirect mortality that occurs after passage through the FCRPS have been observed between barged and ROR migrating Pacific salmon. However, the causes in their annual and seasonal patterns remain unclear. Passage experience can affect survival capacity (or delayed mortality), and I examine this through: a survival challenge at increased water temperature (Subchapter A), a surrogate endpoint challenge (Subchapter B), and indices of fish condition and proximate composition (Subchapter C).

Introduction

Survival capacity of individuals depends on their past experiences (see Chapter 1 for further descriptions). This is particularly important for 10 of the 31 Endangered Species Act (ESA)–listed juvenile salmonid evolutionary significant units (ESUs) of the U.S. west coast (NMFS 2011) that migrate through all or part of the Federal Columbia River Hydropower System (FCRPS; Washington and Oregon). Juveniles from these 10 ESUs migrate to the ocean from different origins, pass through a different numbers of reservoirs and dams, and have travel times and arrival times to the ocean that vary (Ferguson et al. 2005, Williams et al. 2005, Muir et al. 2006, Williams 2008, Muir and Williams *In press*). Various mitigation strategies aimed at reducing direct and indirect mortality exist at each dam. At the crux of this highly managed and anthropogenically modified ecosystem is determining the relative effectiveness of the mitigation strategies in the context of passage experience and ultimately survival.

Two general categories of juvenile fish exist: run-of-river (ROR) and transported fish. Transported fish are intercepted from turbines, removed from the river, placed into barges, and hauled to a release site downstream of Bonneville Dam (BON), the final mainstem dam in the hydropower system. One mitigation strategy is the Juvenile Fish Transportation Program (U.S. Army Corps of Engineers) which collects and barges juvenile salmonids from Lower Granite Dam (LGR), Little Goose Dam (LGS), Lower Monumental Dam (LMN), and McNary Dam (MCN). Barged fish consist of two major groups: 1) fish intercepted from turbines and bypassed through pipes to tailraces of dames through a juvenile bypass system rather than putting them into barges, 2) fish not collected at dams because they pass under turbine intake screens or pass through non-turbine routes. Because of screening efficiency, most fish in this group pass through spillways. For Snake River spring/summer Chinook salmon, barging from LGR has resulted in very high survival rates (98%) (Budy et al. 2002, McMichael et al. 2010a) through the

hydropower system in comparison to ROR passage (approximately 50%) (Williams et al. 2005, Tuomikoski et al. 2010). If transportation effectively mitigated for losses of juveniles passing through reservoirs and dams, and since transported juveniles survive at nearly double the rate of ROR fish, we would expect to see twice the smolt-to-adult return rate (SAR) from transported fish compared to ROR fish. However, this is often not the case. To examine this further, the post-FCRPS SAR of barged and ROR fish has been estimated as a ratio termed differential delayed mortality (*D*) (Peters and Marmorek 2001, Williams et al. 2005). A value of 1 denotes no difference in post-FCRPS SAR, while a value greater (or less) than 1 indicates positive (or negative) effects of transportation on post-FCRPS SAR of barged fish relative to that of ROR fish. Among 56 annual estimates of *D* (with 90% confidence intervals) from 1997 to 2008 for various stocks of hatchery spring/summer Chinook originating from the Snake River Basin, 14 estimates were less than 1, 36 estimates were approximately 1, and 6 were greater than 1. For the wild counterparts, annual estimates from 1994 to 2008 indicated that in 8 out of these 15 years, *D* was less than 1 and in the other 7 years, *D* was approximately 1(Tuomikoski et al. 2010). Also, there is sometimes a seasonal increase in *D* (Anderson et al. 2005, NOAA 2010). Detecting conditions that result in *D* less than 1 on a year-to-year (or annual) and within-season (or seasonal) basis would be valuable to the management of the fish barge transportation program and of fish migrating through the hydropower system.

Several mechanisms of seasonal (or weekly) patterns of the differential in SARs between barged and ROR fish have been hypothesized. Stress has been hypothesized to accumulate during their FCRPS passage experience and result in decreased energetic condition, elevated predation risk, increased disease susceptibility, and decreased smoltification (Budy et al. 2002). The authors made a case that delayed mortality is related to the hydrosystem based on annual estimates of SARs, but the exact mechanisms are unclear. One major difference in the passage experience of

barged and ROR fish is the travel time through the FCRPS (Williams 2008, Muir and Williams *In press*). With barge transportation being only 36 hours in duration from LGR to below BON, and ROR migration down the FCRPS being approximately 4 weeks early in the season and 2 weeks late in the season, many differences in fish quality between barged and ROR fish can occur. Schreck et al. (2006) suggested that differential avian predation from large breeding colonies in the estuary occurred among freshwater surface-oriented Chinook salmon infected with *Renibacterium salmoninarum* and possessing low levels of a smoltification index. Although disease has been hypothesized to affect post-FCRPS survival of these fish, the diverse patterns of pathogen prevalence and survival in disease challenge studies of barged and ROR fish reflect the complexity of pathogen–host–environment relationships (Williams 2001, Arkoosh et al. 2006, Dietrich et al. 2008, Dietrich et al. 2011). Recent estimates of prolonged travel time and decreased survival in the estuary, especially among barged fish early in the season (Eder et al. 2009a, McMichael et al. 2010b), support the hypothesis of differential levels of smoltification and avian predation risk. Also, barged fish lose the opportunity to grow, and are more susceptible to freshwater and marine fish predation after hydrosystem passage (Muir et al. 2006). Strong seasonal patterns of post-FCRPS survival (Schreck et al. 2006) and SARs (Scheuerell et al. 2009) have been observed in ROR fish. The "match/mismatch" of the fish's arrival timing to the ocean when food resources are abundant through upwelling has also been hypothesized as a driver of *D* patterns (Scheuerell and Williams 2005, Scheuerell et al. 2009). Overall, stress, travel time, smoltification, disease, growth, predation, and arrival timing to the estuary and ocean have been hypothesized to affect *D*.

Techniques to estimate delayed mortality after FCRPS passage have involved mark-recapture studies in the field, challenge studies in the laboratory, and snapshot data of fish and environmental conditions. In the field setting, SARs (Marsh et al. 2010a, Tuomikoski et al. 2010)

and survival through specific reaches, such as the lower Columbia River and Estuary (Schreck et al. 2006, Eder et al. 2009a, McMichael et al. 2010b) and the coastal ocean (Rechisky et al. 2009, Porter et al. 2010), have provided the closest true estimates. However, these estimates are only available thanks to great amounts of effort, especially when SARs average 1% and can be as low as 0.07% (Tuomikoski et al. 2010). In contrast, challenge experiments are advantageous in that they require relatively less effort, can occur on a shorter time scale, and can test specific mechanisms of survival capacity. Some examples of challenge experiments are as follows: Overall fish health has been estimated from measures of disease susceptibility in challenges of exposure to *Listonella anguillarum* at concentrations that cause 50% mortality in a 9- or 10-day period (LC50) (Arkoosh et al. 2006, Dietrich et al. 2011). In a challenge of sprint swimming performance, yearling Chinook salmon collected at BON were exhausted more quickly than those collected at LGR (Fryer 2008). Size-selective predation by Northern pikeminnow (*Ptychocheilus oregonensis*) has been found to occur with a difference of at least 10 mm among yearling Chinook salmon (Mesa et al. 2008). Combinations of stressors and performance tests include the effects of an acute thermal stress on the direct mortality, predation risk and physiological responses of juvenile fall Chinook salmon (Mesa et al. 2002). Another study tested the effects of bacterial infection (*Renibacterium salmoninarum*) and physical stress on physiological stress responses, disease progression, and direct mortality (Mesa et al. 2000). Collectively, these studies show that: 1) survival capacity (or health) is greater among barged fish than ROR fish (Arkoosh et al. 2006, Dietrich et al. 2011), 2) with disease, downstream of the barge release site, barged fish experience higher rates of mortality than ROR fish (Dietrich et al. 2011), and 3) single events of stress only showed increased physiological stress responses, but cumulative and chronic effects of stress could lead to mortality (Mesa et al. 2000, Mesa et al. 2002). In order to avoid speculation of cumulative effects on mortality, one could test for survival capacities in survival challenge

experiments that involve the mortality or surrogate endpoints for whole samples. Furthermore, these indices of survival rates can be related to snapshot data of environmental and fish conditions that include water temperature, stress indices, pathogen prevalence, and biological tissue composition.

The overarching goal of this Chapter 4 study is to estimate the relative survival capacity of barged and ROR migrating hatchery yearling Chinook salmon after passage through the FCRPS. In the first set of experiments, I used time to spontaneous mortality and loss of equilibrium in a survival challenge of increased water temperature without food as an index to survival capacity. In the second set of experiments, I tested loss of equilibrium in an anesthetic dose of tricaine methanesulfonate (MS-222) as a surrogate endpoint. In the last set of experiments, I examined non-lethal measures of biological tissue composition. I then compared the survival capacity and biological measures to indices of FCRPS passage experience.

Methods

Field Sampling

Weekly samples of barged and ROR hatchery juvenile spring/summer Chinook salmon were collected on an approximately weekly basis during their outmigration season (Table 4.1). Barged Chinook salmon were subsampled from the Smolt Monitoring Program conducted by the Washington Department of Fish and Wildlife at the Lower Granite Dam Juvenile Fish Monitoring Facility (JFMF) from 24 April to 29 May 2008 and from 9 April to 29 May 2009 and at the Lower Monumental Dam JFMF from 9 April to 29 May 2009. The Chinook salmon were placed in net pens (3ft × 3ft × 4ft) in barge holds and transported for approximately 1.5 days to Bonneville Dam where they were recollected for experimentation. The barged salmon were removed from barge holds into 114-liter plastic bins half-full of water with a battery-powered aerator. At the BON Navigation Lock, the bins were transferred from the barges to a truck and transported 6.5 km to the BON JFMF. Run-of-river (ROR) migrating Chinook salmon were subsamples of the Smolt Monitoring Program conducted by the Pacific States Marine Fisheries Commission at the BON JFMF from 21 April to 2 June 2008 and from 12 April to 5 June 2009. One group of Chinook salmon with passive integrated transponder tags was sub-sampled 17 May 2008 from a study by the National Marine Fisheries Service (courtesy of Douglas M. Marsh, William D. Muir, and John G. Williams) with known origins from above Lower Granite Dam to compare with all the other run-at-large samples of ROR Chinook salmon.

Table 4.1. Samples of barged and ROR migrating spring/summer Chinook salmon collected at Bonneville Dam.

Treatment group	Year	Sample name	Collection date at BON	N
Run-of-River	2008	ROR1.08	4/21/2008	84
		ROR2.08	4/28/2008	84
		ROR3.08	5/7/2008	84
		ROR4.08	5/16/2008	84
		ROR5.08 (PIT-tagged)	5/17/2008	84
		ROR6.08	5/20/2008	84
		ROR7.08	5/25/2008	84
		ROR8.08	6/2/2008	84
	2009	ROR1.09	4/12/2009	83
		ROR2.09	4/19/2009	85
		ROR3.09	4/26/2009	84
		ROR4.09	5/1/2009	84
		ROR5.09	5/9/2009	84
		ROR6.09	5/16/2009	85
		ROR7.09	5/23/2009	84
		ROR8.09	5/30/2009	81
		ROR9.09	6/5/2009	84
Barged from Lower Granite Dam	2008	LGR-B1.08	4/18/2008	85
		LGR-B2.08	4/25/2008	82
		LGR-B3.08A	5/2/2008	84
		LGR-B3.08B	5/2/2008	84
		LGR-B4.08A	5/9/2008	84
		LGR-B4.08B	5/9/2008	84
		LGR-B5.08	5/16/2008	85
		LGR-B6.08	5/23/2008	78
		LGR-B7.08	5/30/2008	84
	2009	LGR-B1.09	4/10/2009	88
		LGR-B2.09	4/17/2009	82
		LGR-B3.09	4/24/2009	86
		LGR-B4.09	5/1/2009	83
		LGR-B5.09	5/10/2009	84
		LGR-B6.09	5/16/2009	84
		LGR-B7.09	5/23/2009	80
		LGR-B8.09	5/30/2009	85
Barged from Lower Monumental Dam	2009	LMN-B5.09	5/10/2009	84
		LMN-B6.09	5/16/2009	84
		LMN-B7.09	5/23/2009	84
		LMN-B8.09	5/30/2009	46

Laboratory Experimentation

2008

After collection, the salmon were anesthetized in MS-222 (75 mg/L buffered to pH of 7.0 with $NaHCO_2$), weighed on an electric balance to 0.01 g precision, and transferred to the 227-liter test tanks for two to three days of acclimation without food. The test tanks were flow-through

with river-water. The salmon were then introduced to the challenge of increased water temperature (24°C) in the absence of food (Subchapter 4A).

2009

After collection, the LGR-barged salmon were allowed to acclimate for 1 to 3 days (on average 1.75 days), LMN-barged salmon 1 to 4 days (on average 2.5 days), and ROR salmon 1 to 3 days (on average 2.2 days) before being tested in trials of LOE in an anesthetic dose of MS-222 (40 $mg \cdot L^{-1}$). Please see Subchapter 4B for the report on the MS-222 LOE experiment. Immediately after each MS-222 LOE trial, the salmon were measured for mass and fork length, and also a subsample for biological impedance analysis (BIA). Please see Subchapter 4C for the report on mass, fork length, condition factor, and BIA-derived proximate compositions. After 3 to 5 days of acclimation since these measurements, the challenge trials at increased water temperature without food were initiated (Subchapter 4A).

Subchapter 4A

Post-Hydropower System Survival Capacity of Barged and ROR Fish Estimated from Challenges of Increased Water Temperature

Abstract

In a heavily anthropogenically modified environment, such as the Federal Columbia River Power System (FCRPS), determining the effectiveness of a mitigation strategy and its related biological and ecological processes on survival are important in the management of ESA-listed species. In this study, I used survival challenge experiments to estimate post-hydropower system survival capacity of fish that had experienced different types of passage through the FCRPS. Challenges were at increased water temperature without food on weekly samples of barged and ROR migrating hatchery yearling Chinook salmon. Measures of survival capacity and heterogeneity were related with fish migration experience (water temperature at barge loading site or after ROR FCRPS migration, T_{site}; a presence/absence index of barging, B; a source of origin index, P_{LGR} and P_{MCN}; body mass, M; and a year index, Y) in an analysis of model selection and multi-model inference based on the corrected Akaike information criterion (AICc). A subset of paired samples barged from Lower Granite Dam (LGR) and Lower Monumental Dam (LMN) were also analyzed (T_{site}; barging site, B_{LGR}; condition factor, CF; P_{LGR}). Also, the seasonal pattern of the ratio of the barged to ROR fish survival capacity measures m_{Barged}:m_{ROR} (where m is the average time to mortality in the challenge experiments) was determined by using the best model from the analyses of model selection and multi-model inference. Finally, an exploratory analysis of how much survival capacity ROR fish lose during FCRPS passage was also conducted. As water temperature increased, survival capacity decreased and heterogeneity among individuals increased. The type of passage through the FCRPS was an important determinant in what water

temperatures fish experienced. Barged fish experienced lower temperatures and higher survival capacities relative to the ROR fish that were migrating out of the system at equivalent times throughout the season. Annual differences were also detected in survival capacity. When comparing LGR- and LMN-barged fish, a temperature-independent barging effect was also detected. The pattern of m_{Barged}:m_{ROR} was close to 1 early in the season, then increased until about day-of-year 145, and finally declined at the end of the season. The loss in survival capacity among ROR fish during FCRPS passage decreased as their travel time decreased throughout the season. My results suggest that: 1) barging fish is beneficial to post-FCRPS survival capacity, especially at LGR and during the mid-to-late outmigration season, 2) decreasing the water temperature experienced by fish would help increase their post-FCRPS survival capacity, and 3) reducing the FCRPS travel time of ROR fish would also help increase their survival capacity. Because of the limited scope of this study, caution is encouraged when comparisons of these results are made to the patterns of post-FCRPS smolt-to-adult return rates of barged and ROR fish.

Introduction

Challenge experiments are valuable tools in studying survival capacity (see Chapter 1 for further descriptions) and physiological ecology. These include temperature challenges to pinpoint the critical thermal maximum and other temperature tolerances (Paladino et al. 1980, Lutterschmidt and Hutchison 1997), pathogen exposures to evaluate disease susceptibility (Arkoosh et al. 2006, Dietrich et al. 2011), and prey introductions to predators to determine levels of predation risk (Mesa 1994, Relyea 2003, Mesa et al. 2008). Temperature challenges are particularly useful in fishes because the physiological ecology of these poikilotherms is strongly controlled by water temperature (Paladino et al. 1980). Major processes in which water

temperature is influential include growth, metabolism, stress response, behavior, spatial distribution, and survival (Brett 1971, Weetman et al. 1998, Clarke and Johnston 1999, Feder and Hofmann 1999, Brannon et al. 2004, Quigley and Hinch 2006). Challenges at increased water temperature could thus test fish survival capacities that involve many of these biological and physiological processes. Many studies have used temperature challenges to examine the physiological ecology of fishes. More specifically, changes due to starvation, disease, toxicity, ageing at the cellular level, seasonal effects as well as synergistic effects (see Paladino et al. 1980 and Lutterschmidt and Hutchison 1997 for review). In the midst of climate change, increased water temperature is an especially relevant stressor to examine because fish will likely face its impacts in the near future.

In this subchapter, I used increased water temperature in a challenge test to examine the seasonal post-FCRPS survival capacities of yearling Chinook salmon that experienced two different types of downstream passage (ROR and barge transportation). I related the measures of survival capacity and heterogeneity to measures of their passage experience that included environmental and biological factors. A subset of paired LGR-barged and LMN-barged samples was also analyzed. Finally, an exploratory analysis of how much survival capacity ROR fish lose during FCRPS passage was conducted. Together, these investigations showed that water temperature, FCRPS passage type, and ROR travel time influenced the survival capacity of yearling Chinook salmon after passage through the hydropower system.

Methods

Survival Capacity and Heterogeneity of Barged and ROR Fish

Experimentation

After the fish were collected, weighed, and acclimated (Methods from Chapter 4), they were challenged at increased water temperature (24°C) in the absence of food. The water in the test tanks was gradually heated over 72 hours to the target challenge temperature. The fish were observed for mortality and loss of equilibrium (LOE) approximately every half hour between 08:00 to 17:00 and once at night between 21:00 and 23:00. If LOE was observed, the fish were euthanized with tricaine methanesulfonate (MS-222; 250 mg/L buffered to 7.0 with $NaHCO_3$). The time to LOE and time to mortality were assumed to be equivalent because once LOE was observed, mortality appeared to be imminent in these challenge tests. All the fish collected were tested in the challenge experiments, unless they exhibited signs or symptoms indicative of disease (UW IACUC protocol #3382-03), and with the exception of some or all of the fish in three samples (LGR.B1.08, LGR.B2.08, and LGR.B3.08; Figure 4A.1). An electrical failure which disabled the aeration system caused the mortalities in the two former samples. In the latter sample, the fish exhibited some irregular behavior during the acclimation period, and died during water exchanges. For water quality conditions, please see Appendix C.

Table 4A.1. Samples of barged and ROR migrating spring/summer Chinook salmon collected at Bonneville Dam. Sample sizes and wet body masses at BON for ROR and LGR-barged fish and at LMN for LMN-barged fish, as well as sample sizes of fish tested in challenge experiments are reported.

Treatment group	Year	Sample name	Collection date at BON	N (collected)	Average mass in grams (SD[1])	N (challenged)
Run-of-River	2008	ROR1.08	4/21/2008	84	34.6	84
		ROR2.08	4/28/2008	84	33.7	84
		ROR3.08	5/7/2008	84	29.4	82
		ROR4.08	5/16/2008	84	28.8	83
		ROR5.08 (PIT-tagged)	5/17/2008	84	27.8	77
		ROR6.08	5/20/2008	84	31.8	84
		ROR7.08	5/25/2008	84	33.4	76
		ROR8.08	6/2/2008	84	31.3	81
	2009	ROR1.09	4/12/2009	83	33.6 (13.7)	79
		ROR2.09	4/19/2009	85	29.2 (7.1)	59
		ROR3.09	4/26/2009	84	31.6 (14.2)	84
		ROR4.09	5/1/2009	84	31.2 (9.4)	19
		ROR5.09	5/9/2009	84	29.9 (9.7)	81
		ROR6.09	5/16/2009	85	29.0 (9.8)	80
		ROR7.09	5/23/2009	84	29.0 (8.6)	82
		ROR8.09	5/30/2009	81	27.4 (4.8)	70
		ROR9.09	6/5/2009	84	31.9 (10.2)	47
Barged from Lower Granite Dam	2008	LGR-B1.08	4/18/2008	85	26.9	0
		LGR-B2.08	4/25/2008	82	27.5	23
		LGR-B3.08A	5/2/2008	84	29.1	81
		LGR-B3.08B	5/2/2008	84	26.9	76
		LGR-B4.08A	5/9/2008	84	30.9	82
		LGR-B4.08B	5/9/2008	84	28.1	82
		LGR-B5.08	5/16/2008	85	30.3	84
		LGR-B6.08	5/23/2008	78	27.1	75
		LGR-B7.08	5/30/2008	84	30.5	81
	2009	LGR-B1.09	4/10/2009	88	27.2 (14.1)	72
		LGR-B2.09	4/17/2009	82	27.1 (8.2)	84
		LGR-B3.09	4/24/2009	86	33.5 (10.2)	0
		LGR-B4.09	5/1/2009	83	26.1 (8.1)	80
		LGR-B5.09	5/10/2009	84	25.6 (6.8)	74
		LGR-B6.09	5/16/2009	84	26.1 (5.4)	71
		LGR-B7.09	5/23/2009	80	26.6 (12.1)	78
		LGR-B8.09	5/30/2009	85	22.4 (5.0)	76
Barged from Lower Monumental Dam	2009	LMN-B5.09	5/10/2009	84	24.9 (5.8)	71
		LMN-B6.09	5/16/2009	84	32.7 (10.4)	71
		LMN-B7.09	5/23/2009	84	27.8 (4.7)	63
		LMN-B8.09	5/30/2009	46	29.3 (5.6)	44

[1] Standard deviation (SD) of mass only available for samples in which individuals were weighed individually. Standard deviation of mass not available for samples in which individuals were bulk weighed.

Dependent Variables

The time at mortality (or surrogate endpoint LOE) from the time that the target temperature was reached in the challenge experiment was recorded for each fish. The average time to mortality (*m*) was calculated. Also, survival curves were fitted by a vitality model (see Methods of Chapter 2 for details about vitality model; Anderson 2000, Salinger et al. 2003) and

vitality parameters *r* (vitality loss rate) and *s* (variability in *r*, also termed evolving heterogeneity of vitality) were estimated with the extrinsic mortality rate (*k*) constrained between 0 and 0.05. Five of the N samples failed to fit the vitality model because of their step-shaped survival curve patterns and were excluded.

Explanatory Variables

The explanatory factors examined included water temperature at barge-loading site or at collection site after ROR passage (T_{site}), barge index (B), LGR-barged index (B_{LGR}), year index (Y), wet body mass (M) measured at Bonneville Dam, condition factor (CF) and the proportions of each sample by three major sources of origin ($\hat{P}_{LGR}$, $\hat{P}_{MCN}$, $\hat{P}_{BON}$) (Appendix C.3). Other explanatory factors were explored, including FCRPS travel time and degree days, but were eliminated due to high correlations with currently examined explanatory factors (Appendix C.2). Some of the likely interactions between variables were also not included (Appendix C.4). The T_{site} index can be thought of as the water temperatures fish have experienced up to the time of sampling (i.e. water temperatures at LGR and LMN for barged fish, and at BON for ROR fish). The water temperatures were "Temperature (WQM)" data recorded at water quality monitoring stations (USACE, NWD), accessed via Columbia Basin Research Data Access in Real Time (CBR DART; cbr.washington.edu/dart). The barge index was 0 for ROR fish and 1 for barged fish. The LGR-barged index was 0 for samples barged from LMN and 1 for those from LGR. The year index was 0 for 2008 and 1 for 2009. Wet body mass was measured to the nearest 0.1 gram. For each sample, the proportion of ROR fish by the first of three select FCRPS dams they encountered (LGR, MCN, or BON) was estimated from travel times and source of origin (hydrologic unit code, HUC) of all PIT-tagged, hatchery, spring-summer Chinook salmon in the Passive Integrated Transponder Tag Information System database (PTAGIS; ptagis.org). All PIT-

tagged hatchery spring/summer Chinook yearlings passing BON on day of sampling ± 1 day were queried for source of origin determined by HUC. The HUC was used to determine which of the three select FCRPS dams the fish encountered (i.e. LGR, MCN, or BON). The number of individuals in each of these three groupings was then used to calculate proportions:

$$\hat{P}_{LGRi} = \frac{n_{LGRi}}{N_i}$$

$$\hat{P}_{MCNi} = \frac{n_{MCNi}}{N_i}$$

$$\hat{P}_{BONi} = \frac{n_{BONi}}{N_i}$$

Where P = proportion
i = sample
N = total number of PIT-tagged hatchery SS Chinook that passed BON on day of sampling ± 1 day
n = number of PIT-tagged SS Chinook, by the first of three select FCRPS dam encountered, on day of sampling ± 1 day

Furthermore, these P estimates were corrected for the proportion of salmon that were ROR and barged hatchery spring-summer Chinook salmon from the NOAA estimation memoranda "Estimation of Percentages for Listed Pacific Salmon and Steelhead Smolts Arriving at Various Locations in the Columbia River Basin in 2008" from John W. Ferguson to James H. Lecky dated January 26, 2009 and "Estimation of Percentages for Listed Pacific Salmon and Steelhead Smolts Arriving at Various Locations in the Columbia River Basin in 2009" from John W. Ferguson to James H. Lecky dated October 15, 2009.

Data Analysis

The dependent variables (m, r, and s) were tested for normality by the Shapiro-Wilk test, and ln-transformed to meet the assumption of normality. Also, the log transformation follows a biologically mechanistic basis with temperature (Anderson et al. 2008). General multi-linear

models were examined to determine the 95% confidence set of best models, the AICc weighting of each explanatory variable, and the model averaging for each parameter (Burnham and Anderson 2002, Mazerolle 2011). Predicted by observed ln(m), ln(r), and ln(s) were graphed for the top four models. The survival capacity measures ln(m) and ln(r) and the heterogeneity measure ln(s) were first analyzed with both samples of barged fish and ROR fish. Because $\hat{P}_{LGR}$, $\hat{P}_{MCN}$, and $\hat{P}_{BON}$ add to 1 (i.e. are complementary), only $\hat{P}_{LGR}$ and $\hat{P}_{MCN}$ were tested. For the analysis with paired LGR- and LMN-barged samples, only ln(m) was examined. I also only examined the explanatory variables T_{site}, CF, B_{LGR}, and P_{LGR}. LMN-barged samples were only collected in 2009, thus the Y index was not necessary. Also, fork lengths used to estimate CF were only collected in 2009, thus M was used in the full analysis with barged fish and ROR fish, and CF was used in the subset analysis with LGR- and LMN-barged fish.

Ratio of survival capacity m_{Barged}:m_{ROR}

To gain insight into the relative survival capacities of barged to ROR fish by the day-of-year they passed LGR, I determined the seasonal pattern of m_{Barged}:m_{ROR} based on the best model that I determined using all barged and ROR samples. The data used for T_{site} in this predictive model of m_{Barged}:m_{ROR} were the "Temperature (WQM)" data recorded daily at water quality monitoring stations (USACE, NWD), accessed via CBR DART. The day-of-year of passage through LGR and BON needed for T_{site} were estimated from travel times of passive integrated transponder (PIT)–tagged fish in the PIT Tag Information System (PTAGIS; ptagis.org).

Survival Capacity Loss During FCRPS Passage of ROR Fish

Dependent and Explanatory Variables

To explore how survival capacity of ROR fish changes from LGR to BON throughout the outmigration season, hypothetical ROR samples at LGR were used to compare to observed ROR samples at BON (Figure 4A.1). The survival capacity $\widehat{m}$ of hypothetical ROR samples were assumed to be equivalent to the observed *m* of LGR-barged samples, because any change in the survival capacity of barged fish during their 1.5 days of passage through the FCRPS was assumed to be negligible. The day-of-year of passage through LGR (DOY_{LGR}) for these hypothetical ROR samples was estimated from travel times of PIT–tagged fish in the PTAGIS database. Furthermore, the observed ROR samples included in the analysis were only those with $\hat{P}_{LGR} > 0.4$ to minimize biases from run-at-large samples but still maintaining a reasonable sample samples size for a linear regression.

Data Analysis

Linear relationships were determined between $\widehat{m}$ and $\hat{DOY}_{LGR}$ for the hypothetical ROR samples, and between *m* and DOY_{BON} for the observed ROR samples. The percent survival capacity loss from LGR to BON of the hypothetical ROR fish was estimated as:

$$\%\ \text{survival capacity loss} = \frac{\widehat{m}_{LGRi} - m_{BONi}}{\widehat{m}_{LGRi}} \times 100.$$

Seven hypothetical samples with DOY_{BON} set at every 10 days starting from DOY 100 (9 May 2008 or 10 May 2009) were estimated from these relationships and compared to travel time from LGR to BON and water temperature at BON (Temperature WQM; USACE from CBR DART).

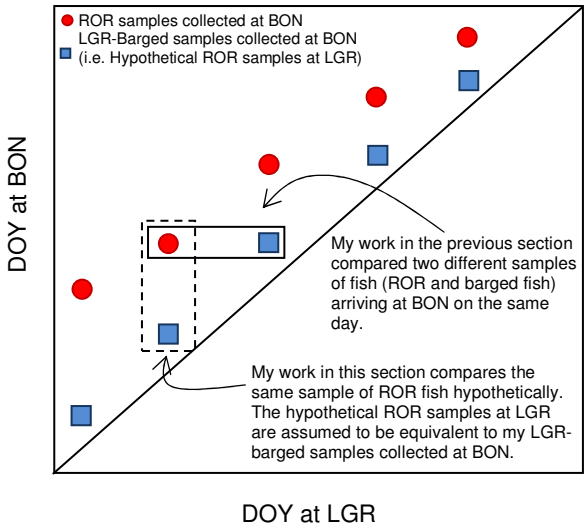

Figure 4A.1. Conceptual diagram of survival capacity loss during ROR passage from LGR to BON (i.e. hypothetical ROR fish samples), based on LGR-barged and ROR fish collected at Bonneville Dam. This is a simple example with conveniently timed samples to elucidate the comparison conceptually. The analysis is based on linear relationships of these samples.

Results

Predictors of survival capacity indices ln(*m*) and ln(*r*)

There is a seasonal decline in the natural log of the average time to mortality, ln(*m*), in the thermal challenges and a significant difference between barged and ROR fish, but not between the two types of barged fish (Figure 4A.2). Water temperature (T_{site}) was present in all models for ln(*m*) and for the natural log of the vitality loss rate, ln(*r*), of both 95% confidence sets (Figures 4A.3 and 4A.5; Table 4A.2–Table 4A.5). There were significant differences in T_{site} by type of FCRPS passage (ANOVA, p=0.002), in which T_{site} experienced by LGR-barged fish was less than that experienced by LMN-barged fish (p=0.036), and also less than that experienced by ROR fish (p=0.001) (see bottom of ln(*m*) vs. T_{site} graph in Figure 4A.3). The year index was the

parameter with the second highest AICc weight in the ln(m) models (Table 4A.3). The year index indicated higher survival capacity in 2009 than in 2008. The AICc weight of all the other explanatory variables of the ln(m) and ln(r) models were less than 0.3 (Table 4A.3 and Table 4A.5), their inclusion in the models did not change the coefficient of determination (r^2) by much (Table 4A.2 and Table 4A.4), and their 80% confidence interval included zero (Table 4A.3 and Table 4A.5). In the plots of predicted by observed survival capacity measures, the 1:1 line fell in between a group of models (Figures 4A.4 and 4A.6). Only the top four models were graphed to show that no single model was truly the best with estimated model-averaged parameters, but rather a group of models were among the best. The model with T_{site}, Y and M underestimated ln(m), while the three other graphed models (T_{site} alone or with Y, and with P_{LGR} or P_{MCN}) overestimated ln(m). Likewise, the model with T_{site} and M overestimated ln(r), while the other three models graphed underestimated this survival capacity measure.

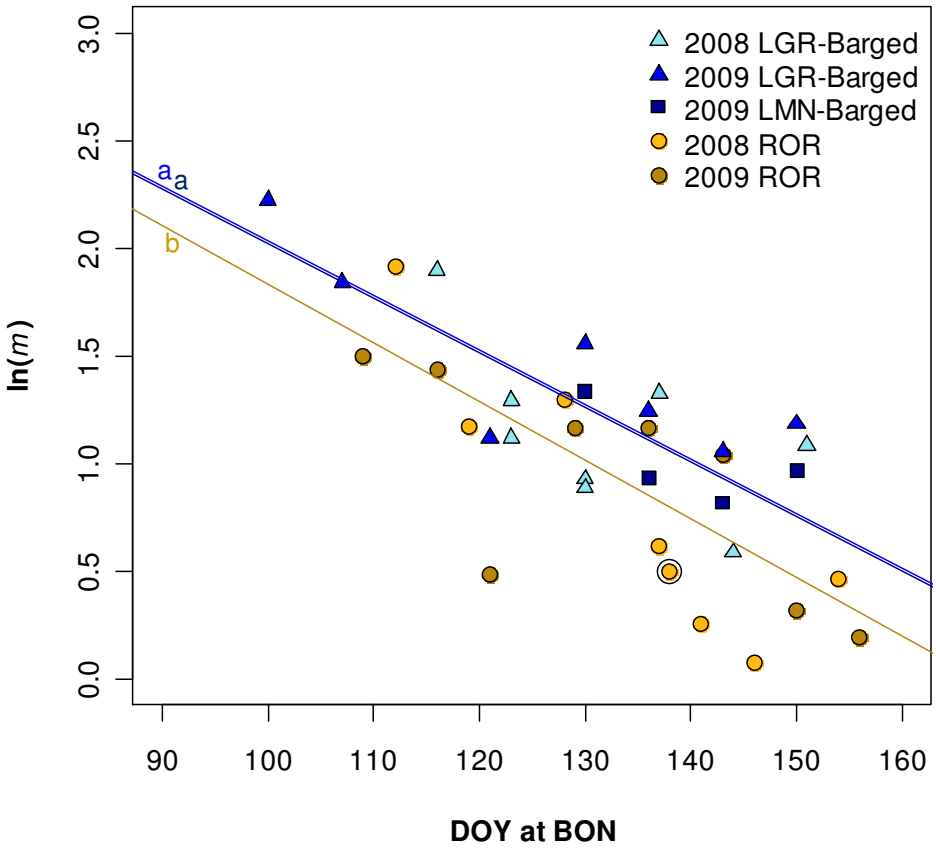

Figure 4A.2. Relationship between the survival capacity ln(*m*) in an increased water challenge experiment without food and the day of year (DOY) of sample collection at Bonneville Dam (BON). The treatment groups tested were juvenile, hatchery, spring/summer Chinook salmon barged from Lower Granite Dam (LGR-barged), barged from Lower Monumental Dam (LMN-barged), and run-of-river (ROR) in two years of study. The circle represents the ROR sample PIT-tagged at LGR.

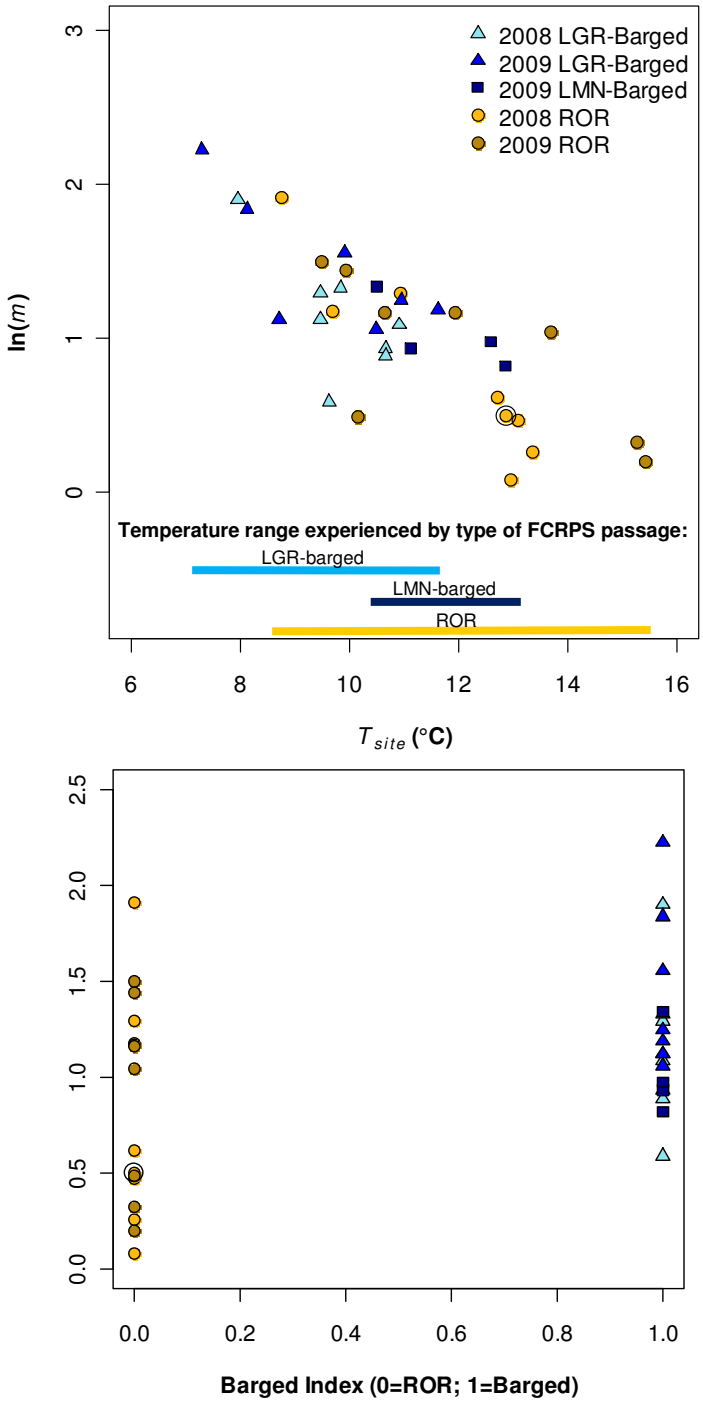

Figure 4A.3. Survival capacity ln(m) of hatchery spring/summer Chinook salmon by the water temperature at barge loading site LGR/LMN or collection site BON after ROR migration (T_{site}) (top graph) and a year index (bottom graph). The fish were collected at Bonneville Dam (Columbia River, Oregon/Washington), and subsequently challenged with increased water temperature without food. The circle represents the ROR sample PIT-tagged at LGR. N=35 samples

Table 4A.2. General multi-linear modeling of ln(m): 95% confidence set with number of parameters (K), difference in bias-corrected AIC score from best model (ΔAICc), AICc weight across all models tested, and coefficient of determination (r^2). All models have an a intercept. Explanatory variables are water temperature at barge loading site LGR/LMN or collection site BON after ROR migration (T_{site}), barge index (B), fish wet mass (M), proportion of sample encountering LGR or MCN as first FCRPS dam (respectively P_{LGR} and P_{MCN}), year index (Y). N=35 samples

Models of ln(m)	K	ΔAICc	AICc Weight	r^2
$T_{site} + Y$	4	0.00	0.298	0.692
$T_{site} + Y + P_{LGR}$	5	2.51	0.085	0.694
$T_{site} + Y + M$	5	2.60	0.081	0.693
$T_{site} + Y + P_{MCN}$	5	2.68	0.078	0.692
$T_{site} + Y + B$	5	2.70	0.077	0.692
T_{site}	3	3.03	0.065	0.639
$T_{site} + M$	4	4.17	0.037	0.653
$T_{site} + Y + M + P_{LGR}$	6	4.87	0.026	0.699
$T_{site} + Y + B + P_{LGR}$	6	5.14	0.023	0.696
$T_{site} + Y + M + B$	6	5.30	0.021	0.695
$T_{site} + Y + P_{LGR} + P_{MCN}$	6	5.42	0.020	0.694
$T_{site} + Y + M + P_{MCN}$	6	5.43	0.020	0.694
$T_{site} + P_{MCN}$	4	5.44	0.020	0.640
$T_{site} + P_{LGR}$	4	5.51	0.019	0.639
$T_{site} + B$	4	5.58	0.018	0.639
$T_{site} + Y + B + P_{MCN}$	6	5.59	0.018	0.693
$T_{site} + M + P_{LGR}$	5	5.71	0.017	0.665
$T_{site} + M + B$	5	6.48	0.012	0.657
$T_{site} + M + P_{MCN}$	5	6.59	0.011	0.656
$T_{site} + B + P_{LGR}$	5	7.53	0.007	0.647

Table 4A.3. General multi-linear modeling of ln(m): Explanatory variable weight by AICc, model-averaged parameter estimates (with unconditional standard errors), 80% confidence interval of parameters, and predicted direction of effect on hatchery spring/summer Chinook salmon survival capacity after passage through the FCRPS.

Variables (units) in Models of ln(m)	Parameter	Variable Weight Across 95% Confidence Set	Estimate (SE)	80% Confidence Interval	Effect on Survival Capacity
T_{site}, Water temperature (°C)	b_{Tsite}	1.000	-0.21 (0.03)	-0.25, -0.17	Negative
Y, Year index	b_Y	0.756	0.23 (0.10)	0.10, 0.36	Higher in 2009 than in 2008
M, Mass (grams)	b_M	0.236	-0.02 (0.02)	-0.05, 0.01	Zero
P_{LGR}, Proportion of sample from above LGR	b_{Plgr}	0.206	-0.12 (0.22)	-0.40, 0.16	Zero
B, Barge index	b_B	0.185	0.02 (0.19)	-0.23, 0.26	Zero
P_{MCN}, Proportion of sample from above MCN	b_{Pmcn}	0.175	0.25 (0.94)	-0.96, 1.46	Zero
Intercept	a	NA	3.42 (0.55)	2.72, 4.12	Positive

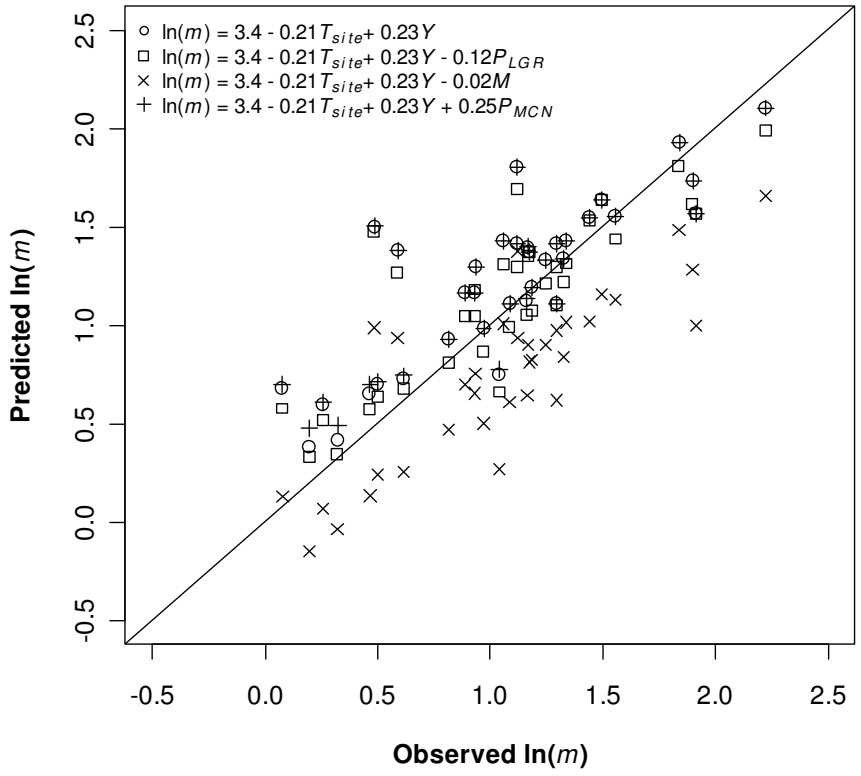

Figure 4A.4. Predicted by observed survival capacity ln(m) for the top four models of the 95% confidence set. N=35

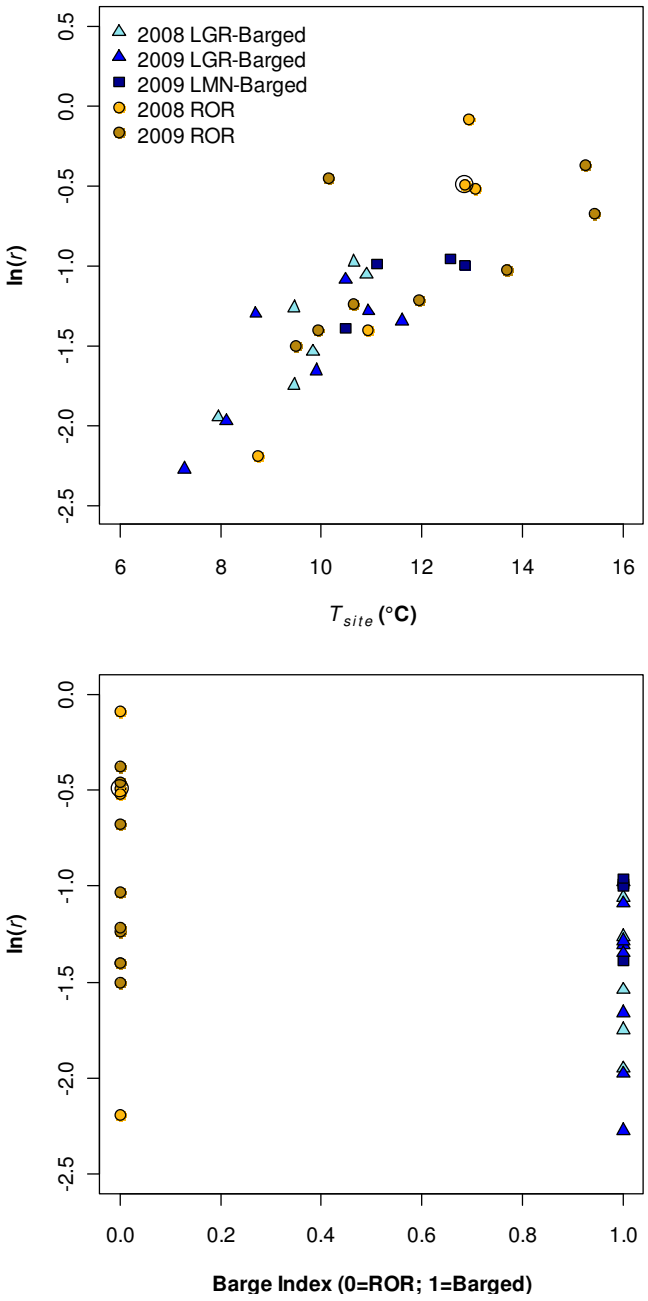

Figure 4A.5. Survival capacity ln(r) of hatchery spring/summer Chinook salmon by the water temperature at barge loading site LGR/LMN or collection site BON after ROR migration (T_{site}) (top graph) and barge index (bottom graph). The fish were collected at Bonneville Dam (Columbia River, Oregon/Washington), and subsequently challenged with increased water temperature without food. Note that r is the vitality loss rate and thus follows an opposite pattern compared to the average time to mortality (m). The circle represents the ROR sample PIT-tagged at LGR. N= 30 samples

Table 4A.4**.** General multi-linear modeling of ln(*r*): 95% confidence set with number of parameters (K), difference in bias-corrected AIC score from best model (ΔAICc), AICc weight across all models tested, and coefficient of determination (r^2). All models have an *a* intercept. Explanatory variables are water temperature at Bonneville Dam (*T*), barge index (*B*), fish wet mass (*M*), proportion of sample encountering LGR or MCN as first FCRPS dam (respectively P_{LGR} and P_{MCN}), year index (*Y*). N= 30 samples

Models of ln(*r*)	K	ΔAICc	AICc Weight	r^2
T_{site}	3	0.00	0.208	0.636
$T_{site} + M$	4	1.62	0.093	0.648
$T_{site} + P_{MCN}$	4	1.70	0.089	0.647
$T_{site} + Y$	4	1.71	0.088	0.647
$T_{site} + B$	4	2.38	0.063	0.639
$T_{site} + P_{LGR}$	4	2.67	0.055	0.636
$T_{site} + M + P_{MCN}$	5	3.02	0.046	0.666
$T_{site} + Y + P_{MCN}$	5	3.60	0.034	0.659
$T_{site} + B + P_{LGR}$	5	3.73	0.032	0.658
$T_{site} + B + P_{MCN}$	5	3.75	0.032	0.657
$T_{site} + M + P_{LGR}$	5	4.05	0.027	0.654
$T_{site} + M + Y$	5	4.06	0.027	0.654
$T_{site} + B + Y$	5	4.40	0.023	0.650
$T_{site} + M + B$	5	4.52	0.022	0.648
$T_{site} + P_{LGR} + P_{MCN}$	5	4.57	0.021	0.648
$T_{site} + Y + P_{LGR}$	5	4.61	0.021	0.647
$Tsite + B + P_{LGR} + P_{MCN}$	6	4.68	0.020	0.682
$T_{site} + M + Y + P_{MCN}$	6	5.80	0.011	0.670
$T_{site} + M + P_{LGR} + P_{MCN}$	6	5.91	0.011	0.668
$T_{site} + M + B + P_{LGR}$	6	5.96	0.011	0.668
$T_{site} + B + Y + P_{MCN}$	6	6.05	0.010	0.667
$T_{site} + M + B + P_{MCN}$	6	6.07	0.010	0.667

Table 4A.5. General multi-linear modeling of ln(r): Explanatory variable weight by AICc, model-averaged parameter estimates (with unconditional standard errors), 80% confidence interval of parameters, and predicted direction of effect on hatchery spring/summer Chinook salmon survival capacity after passage through the FCRPS (Idaho, Oregon, and Washington). Note that positive values of r relate to negative effects on survival capacity. N=30 samples

Variables (units) in Models of ln(r)	Parameter	Variable Weight Across 95% Confidence Set	Estimate (SE)	80% Confidence Interval	Effect on Survival Capacity
T_{site}, Water temperature (°C)	b_{Tsite}	1.000	0.22 (0.041)	0.16, 0.27	Negative
P_{MCN}, Proportion of sample from above MCN	b_{Pmcn}	0.298	-1.12 (1.06)	-2.49, 0.24	Zero
M, Mass (grams)	b_M	0.270	0.02 (0.02)	-0.01, 0.06	Zero
B, Barge index	b_B	0.234	-0.18 (0.27)	-0.54, 0.17	Zero
Y, Year index	b_Y	0.226	-0.11 (0.13)	-0.27, 0.06	Zero
P_{LGR}, Proportion of sample from above LGR	b_{Plgr}	0.197	0.20 (0.35)	-0.25, 0.64	Zero
Intercept	a	NA	-3.74 (0.67)	-4.60, -2.89	Positive

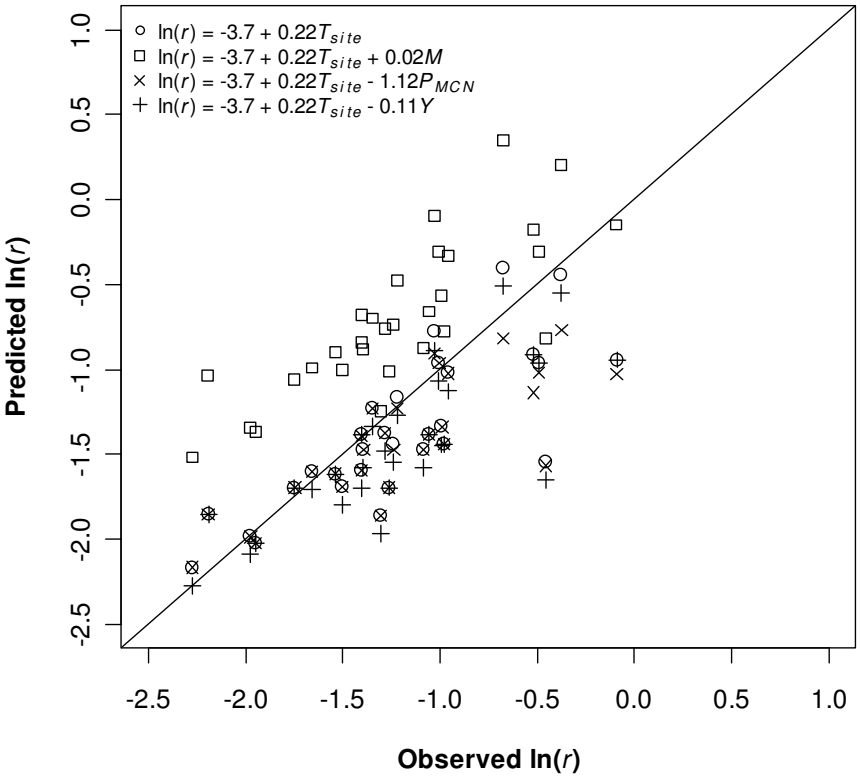

Figure 4A.6. Predicted by observed survival capacity ln(r) for the top four models of the 95% confidence set. Line represents the 1:1 line. N=30 samples

Predictors of heterogeneity among individuals, ln(*s*)

Heterogeneity among individuals was best explained by T_{site} (Figure 4A.7; Table 4A.6 and Table 4A.7). Although it was not present in all the models of the 95% confidence set as was the case in the ln(*m*) and ln(*r*) models, T_{site} still held the greatest AICc weight (Table 4A.7). As water temperature increased throughout the season, heterogeneity among individuals also increased. There was more heterogeneity among individuals in 2008 than 2009. Mass, the barge index, and interestingly the source of origin indices did not significantly affect the heterogeneity among individuals. Interestingly, the PIT-tagged sample had nearly zero heterogeneity among individuals. Less than 35% of the variation in ln(*s*) was explained by the models in the 95% confidence set (Table 4A.6). Again, the predicted survival capacity plotted against the observations showed that no single model but rather a group of models best portrayed the estimated parameters by taking uncertainty into account (Figure 4A.8). Looking at the top four models of the 95% confidence set, three of the models that include T_{site} and *M* or *Y* overestimated survival capacity, and the one with T_{site} , *M* and *Y* underestimated it.

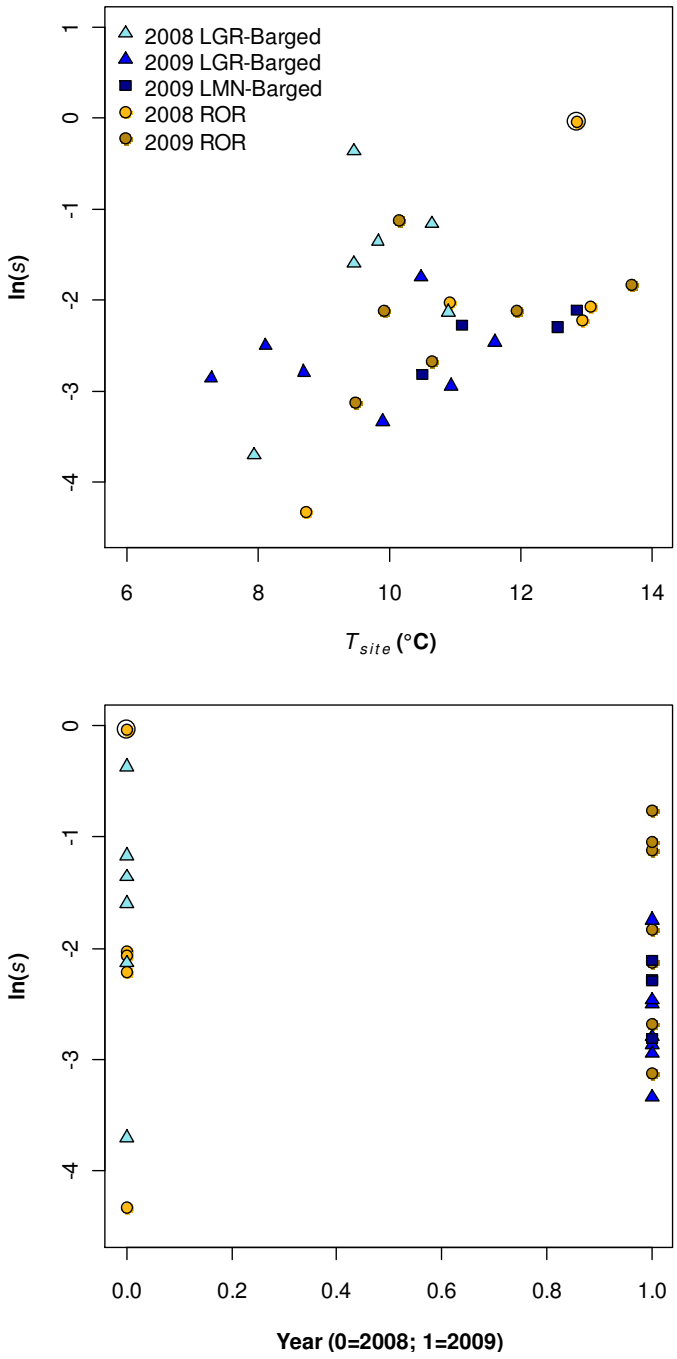

Figure 4A.7. Heterogeneity among individuals ln(*s*) of hatchery spring/summer Chinook salmon by the water temperature at barge loading site LGR/LMN or collection site BON after ROR migration (T_{site}) and a year index. The fish were collected at Bonneville Dam (Columbia River, Oregon/Washington), and subsequently challenged with increased water temperature and without food. The circle represents the ROR sample PIT-tagged at LGR. N=30 samples

Table 4A.6. General multi-linear modeling of ln(s): 95% confidence set with number of parameters (K), difference in bias-corrected AIC score from best model (ΔAICc), AICc weight across all models tested, and coefficient of determination (r^2). All models have an a intercept. Explanatory variables are water temperature at Bonneville Dam (T), barge index (B), fish wet mass (M), proportion of sample encountering LGR or MCN as first FCRPS dam (respectively P_{LGR} and P_{MCN}), year index (Y). N=30 samples

Models of ln(s)	K	ΔAICc	AICc Weight	r^2
T_{site}	3	0.00	0.172	0.246
$T_{site} + Y$	4	0.29	0.149	0.304
$T_{site} + M + Y$	5	1.73	0.073	0.337
$T_{site} + M$	4	2.37	0.053	0.237
$T_{site} + P_{LGR}$	4	2.43	0.051	0.252
$T_{site} + B$	4	2.49	0.050	0.251
$T_{site} + P_{MCN}$	4	2.67	0.045	0.246
$T_{site} + B + Y$	5	2.83	0.042	0.312
$T_{site} + Y + P_{LGR}$	5	2.88	0.041	0.311
$T_{site} + Y + P_{MCN}$	5	3.18	0.035	0.304
P_{MCN}	3	3.72	0.027	0.146
$Y + P_{MCN}$	4	4.72	0.016	0.193
$T_{site} + M + Y + P_{MCN}$	6	4.76	0.016	0.339
$T_{site} + M + Y + P_{LGR}$	6	4.87	0.015	0.337
$T_{site} + B + M + Y$	6	4.87	0.015	0.337
$T_{site} + M + P_{LGR}$	5	5.21	0.013	0.255
$T_{site} + M + P_{MCN}$	5	5.23	0.013	0.255
$T_{site} + B + M$	5	5.24	0.013	0.254
$T_{site} + P_{LGR} + P_{MCN}$	5	5.29	0.012	0.253
$T_{site} + B + P_{LGR}$	5	5.33	0.012	0.252
$T_{site} + B + P_{MCN}$	5	5.33	0.012	0.252
$T_{site} + B + Y + P_{MCN}$	6	5.89	0.009	0.314
$P_{LGR} + P_{MCN}$	4	5.9244	0.0089	0.160
$T_{site} + B + Y + P_{LGR}$	6	5.9822	0.0086	0.312
$T_{site} + Y + P_{LGR} + P_{MCN}$	6	5.9878	0.0086	0.312
$M + P_{MCN}$	4	6.0424	0.0084	0.157
$M + Y + P_{MCN}$	5	6.2715	0.0075	0.228
$B + P_{MCN}$	4	6.3252	0.0073	0.149
$Y + P_{LGR} + P_{MCN}$	5	7.0545	0.0051	0.208
$B + P_{LGR}$	4	7.3509	0.0044	0.119
Y	3	7.459	0.0041	0.033
$B + Y + P_{MCN}$	5	7.4619	0.0041	0.197
B	3	7.6779	0.0037	0.026

Table 4A.7. General multi-linear modeling of ln(s): Explanatory variable weight by AICc, model-averaged parameter estimates (with unconditional standard errors), 80% confidence interval of parameters, and predicted direction of effect on hatchery spring/summer Chinook salmon survival capacity after passage through the FCRPS. N=30 samples

Variables (units) in Models of ln(s)	Parameter	Variable Weight Across 95% Confidence Set	Estimate (SE)	80% Confidence Interval	Effect on Heterogeneity
T_{site}, Water temperature (°C)	b_{Tsite}	0.899	0.24 (0.09)	0.13, 0.36	Positive
Y, Year index	b_Y	0.471	-0.50 (0.33)	-0.93, -0.08	More in 2008 than in 2009
M, Mass (grams)	b_M	0.270	-0.05 (0.07)	-0.14, 0.04	Zero
P_{MCN}, Proportion of sample from above MCN	b_{Pmcn}	0.247	1.84 (3.09)	-2.11, 5.80	Zero
B, Barge index	b_B	0.189	0.04 (0.56)	-0.68, 0.76	Zero
P_{LGR}, Proportion of sample from above LGR	b_{Plgr}	0.188	0.24 (0.67)	-0.62, 1.09	Zero
Intercept	a	NA	-4.00 (1.73)	-6.21, -1.78	Negative

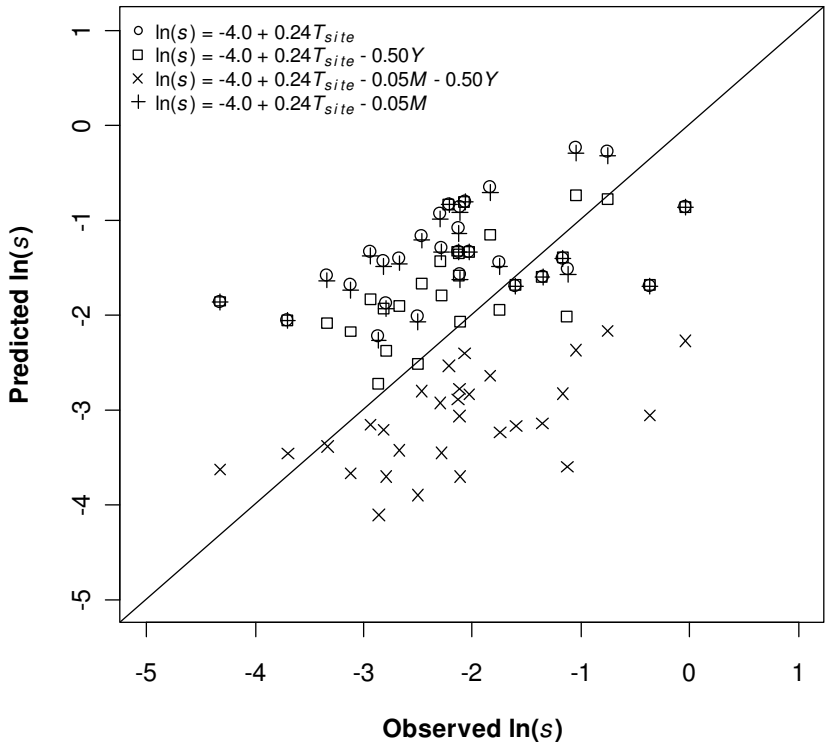

Figure 4A.8. Predicted by observed heterogeneity among individuals ln(s) for the top four models of the 95% confidence set. Line represents the 1:1 line. Each one of these four models is significant with $p < 0.05$. N=30 samples

Survival capacity of LGR-barged and LMN-barged yearling Chinook salmon

The survival capacity ln(m) of paired LGR-barged and LMN-barged samples were statistically different (paired t-test; t=11.2359, df = 3, p=0.0021; Figure 4A.9). However, in the model averaging analysis, T_{site} was still the most important predictor of ln(m) (Table 4A.8 and Table 4A.9). In the three other models of the 95% confidence set, the explanatory variables CF, B_{LGR} and P_{LGR} each had less than 10% of the AICc total weight (Table 4A.9). Fish survival capacity was negatively influenced by water T_{site} and CF. The yearling Chinook salmon barged from LGR had greater survival capacity than those barged from LMN. There was no effect from source of origin being either from above LGR or from between LGR and LMN. When comparing the predicted with the observed estimates of ln(m), the data points closest to the 1:1 line were from the models that included T_{site}, CF or B_{LGR}, but not P_{LGR} (Figure 4A.10). The model with T_{site} underestimates the survival capacity measures, and the models with CF or B_{LGR} overestimates them.

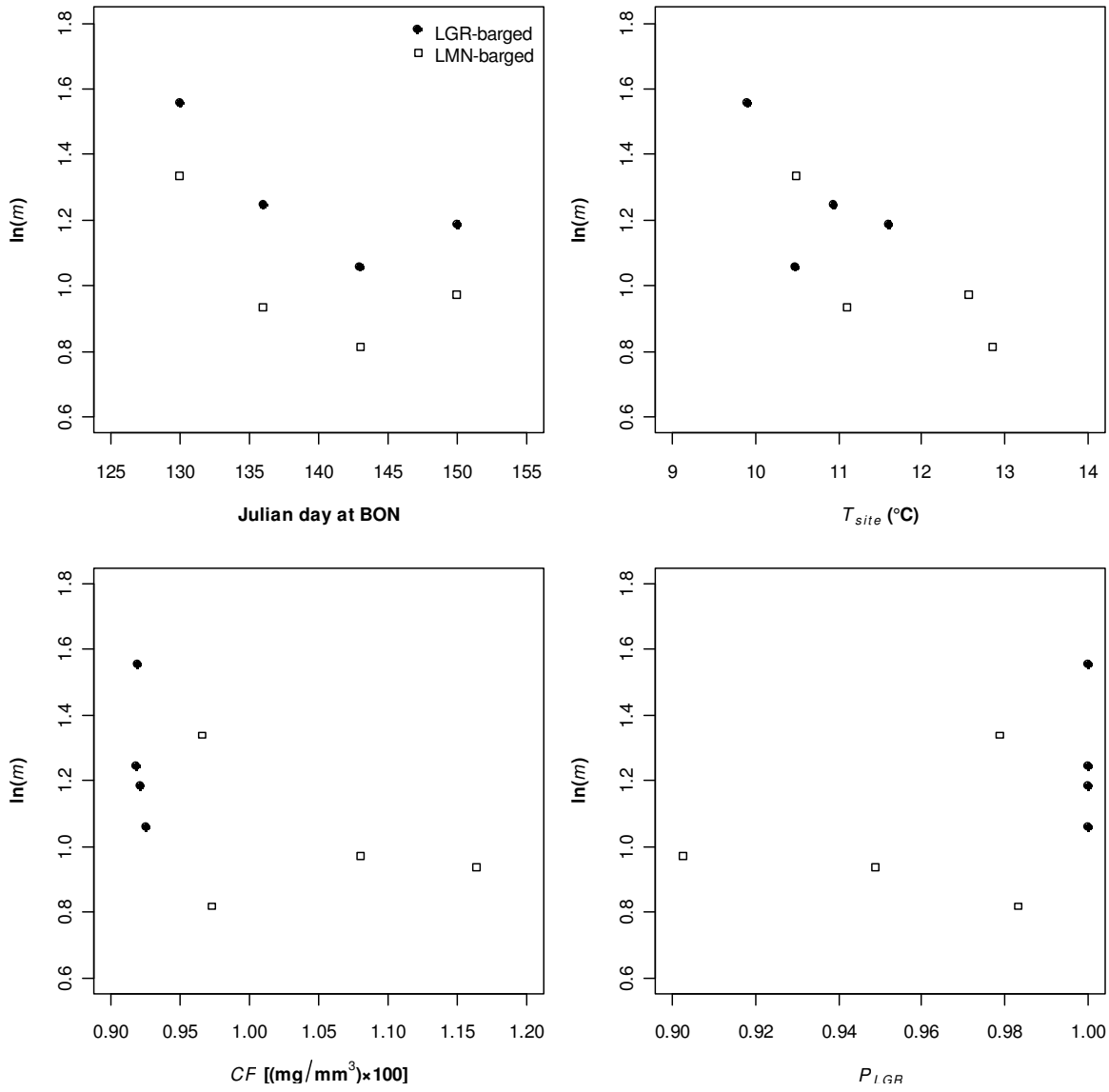

Figure 4A.9. Survival capacity ln(*m*) of samples barged from Lower Granite Dam (LGR-barged) and Lower Monumental Dam LMN (LMN-barged) to Bonneville Dam by four predictor variables. N= 8 samples

Table 4A.8. General multi-linear modeling of ln(m) for paired LGR- and LMN-barged samples: 95% confidence set with number of parameters (K), difference in bias-corrected AIC score from best model (ΔAICc), AICc weight across all models tested, and coefficient of determination (r^2). All models have an a intercept. Explanatory variables are water temperature at barge loading site LGR or LMN (T_{site}), condition factor (CF), LGR-barged index (B_{LGR}), and proportion of sample originating from above LGR (P_{LGR}). N= 8 samples

Models of ln(m)	K	ΔAICc	AICc Weight	r^2
T_{site}	3	0.00	0.776	0.619
CF	3	4.80	0.070	0.306
B_{LGR}	3	4.87	0.068	0.301
P_{LGR}	3	5.70	0.045	0.224

Table 4A.9. General multi-linear modeling of ln(m) for paired LGR- and LMN-barged samples: Explanatory variable weight by AICc, model-averaged parameter estimates (with unconditional standard errors), 80% confidence interval of parameters, and predicted direction of effect on hatchery spring/summer Chinook salmon survival capacity after passage through the FCRPS. N = 8 samples

Variables (units) in Models of ln(m)	Parameter	Variable Weight Across 95% Confidence Set	Estimate (SE)	80% Confidence Interval	Effect on Survival Capacity
T_{site}, Water temperature (°C)	b_{Tsite}	0.809	-0.18 (0.06)	-0.26, -0.11	Negative
CF, Condition factor [(mg/mm^3) × 100]	b_{CF}	0.073	-1.32 (0.91)	-2.48, -0.15	Negative
B_{LGR}, LGR-barged index	b_{Blgr}	0.071	0.23 (0.16)	0.02, 0.44	Positive
P_{LGR}, Proportion of sample from above LGR	b_{Plgr}	0.047	2.83 (2.70)	-0.63, 6.30	Zero
Intercept	a	NA	2.77 (1.48)	0.86, 4.67	Positive

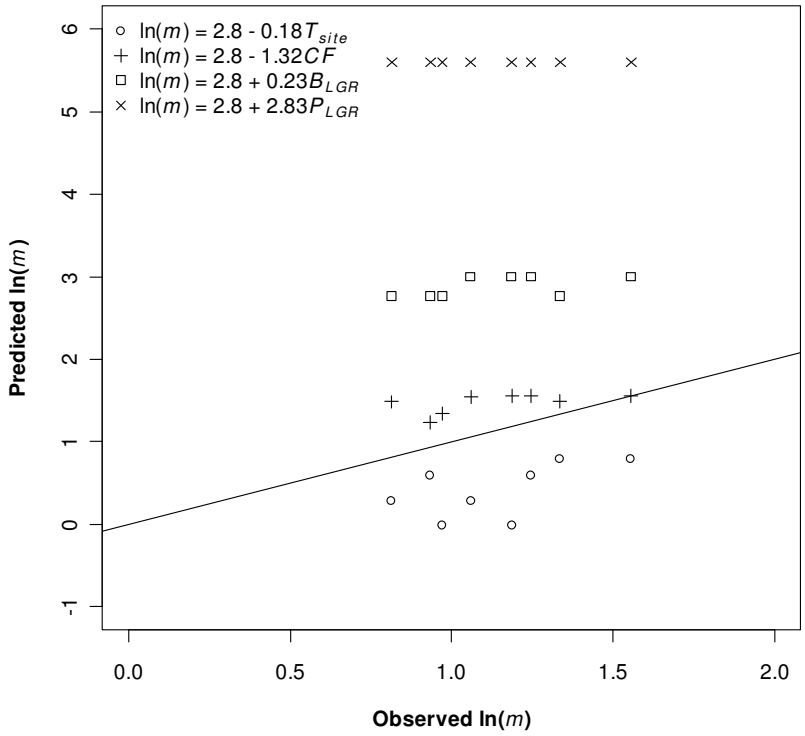

Figure 4A.10. Predicted by observed survival capacity ln(*m*) of paired barged samples for the models in the 95% confidence set. Line represents the 1:1 line. N=8 samples

Ratio of survival capacity m_{Barged}:m_{ROR}

The ratio of survival capacity m_{Barged}:m_{ROR} showed a seasonal pattern that remained close to 1 early in season, that then generally increased until approximately DOY 145 (24 May 2008 and 25 May 2009), and finally decreased during the rest of the outmigration season. The averages ± standard deviations (95% confidence intervals) of the predicted m_{Barged}:m_{ROR} in 1–30 April, 1–31 May, 1–15 June, and 1 April–15 June 2008 were respectively 1.08 ± 0.11 (0.86–1.30), 1.59 ± 0.40 (0.81–2.37), 1.66 ± 0.19 (1.29–2.03), and 1.40 ± 0.38 (0.66–2.14). Those in 2009 were respectively 1.19 ± 0.15 (0.90–1.48), 1.43 ± 0.33 (0.78–2.08), 1.65 ± 0.19 (1.28–2.02), and 1.38 ± 0.30 (0.79–1.97).

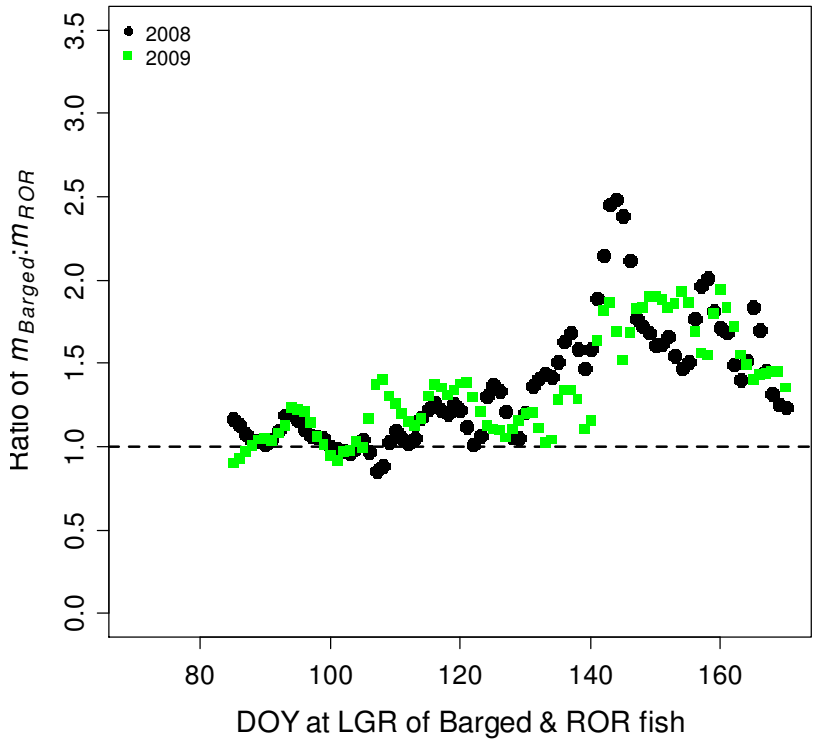

Figure 4A.11. Ratio of survival capacity measures m_{Barged}:m_{ROR} from challenge experiments at increased water temperature in the absence of food. The m_{Barged}:m_{ROR} predicted model was determined from the best model of the 95% confidence set of the general multi-linear modeling with weighted averaged parameters: $\frac{m_{Barged}}{m_{ROR}} = \frac{\exp(3.42 - 0.21(Tsite_{Barged,x}))}{\exp(3.42 - 0.21(Tsite_{ROR,x}))}$.

Survival Capacity Loss During FCRPS Passage of ROR Fish

ROR travel time decreased throughout the season (Figure 4A.11A). The relationship between DOY_{LGR} and DOY_{BON} (Figure 4A.11B) was used to determine the times of passage of the hypothetical samples at LGR (Figure 4A.12). Survival capacity *m* decreased throughout the season for the hypothetical ROR samples at LGR and the observed ROR samples at BON (Figure 4A.13). The percent survival capacity loss was greatest early in the season when travel times were the longest (Figures 4A.13 and 4A.14). There was a 41% survival capacity loss between LGR and BON for the hypothetical ROR sample #1 passing LGR on DOY 82 (22 March 2008 or 23 March 2009), a 20% drop for the hypothetical ROR sample #6 on DOY 137 (16 May 2008 or 17 May 2009), and a 14% increase for the hypothetical sample #7 on DOY 146 (25 May 2008 or 26 May 2009). When travel time from LGR to BON was less than 13 days, the percent survival capacity *m* increased during FCRPS passage (Figure 4A.14). Despite this increase late in the outmigration season, the overall seasonal pattern of survival capacity is negative. The survival capacity $\hat{m}$ of hypothetical ROR samples at LGR was only 12% late in the season on DOY 147 compared to what it was in the early season on DOY 78. At BON, survival capacity *m* was 24% on DOY 160 compared to what it was in the early season on DOY 100 (April 10).

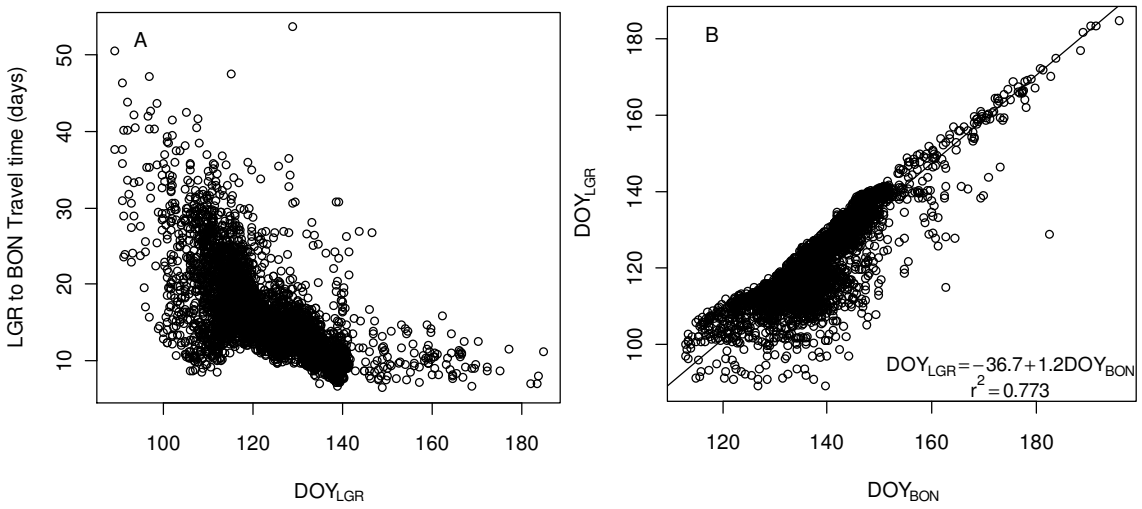

Figure 4A.12. (A) Travel time from LGR to BON by DOY_{LGR} and (B) DOY_{LGR} by DOY_{BON} for hatchery juvenile spring/summer Chinook salmon in 2008 and 2009. The data are LGR-released fish from the PTAGIS database.

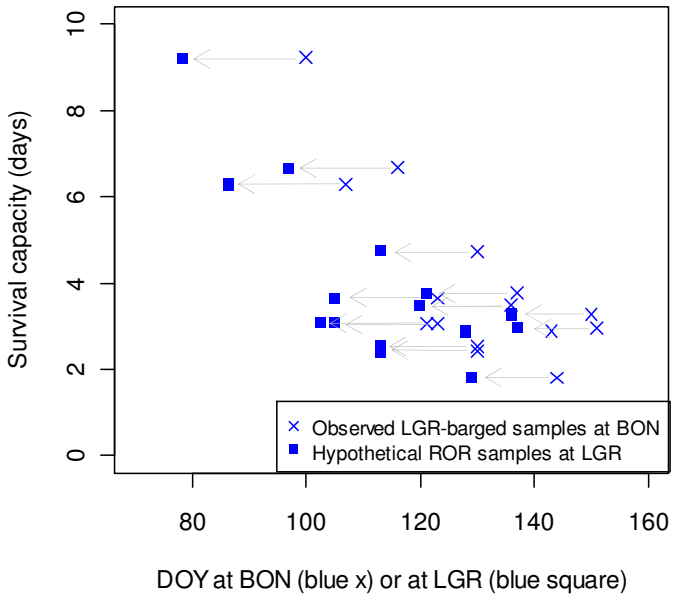

Figure 4A.13. Survival capacity of LGR-barged juvenile hatchery spring/summer Chinook salmon at BON and depiction of these samples as hypothetical ROR fish at LGR based on ROR travel times of these counterparts in the PTAGIS database.

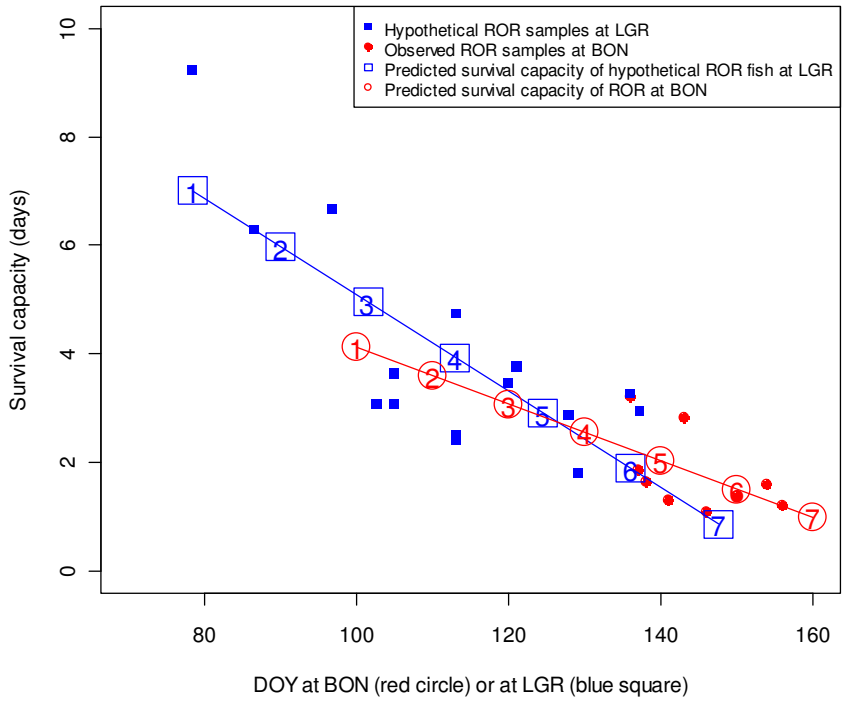

Figure 4A.14. Survival capacity of juvenile, hatchery, spring/summer Chinook salmon, measured as the average time to mortality (*m*) in challenge trials of increased water temperature without food for ROR samples collected at Bonneville Dam and hypothetical ROR samples at LGR. N = 35 samples

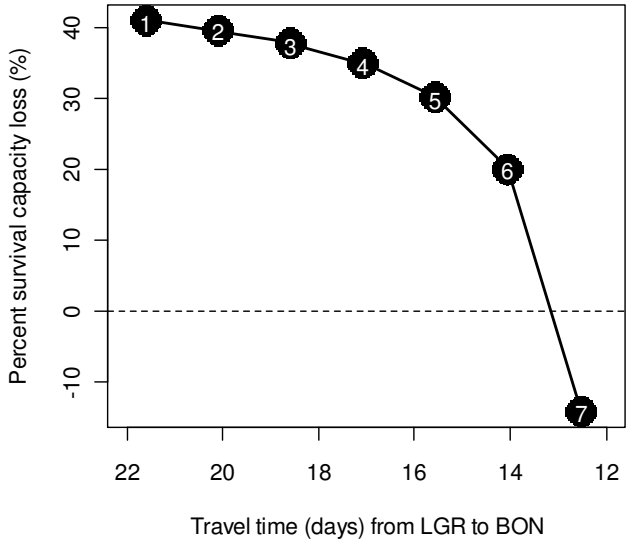

Figure 4A.15. Estimated percent survival capacity loss from LGR to BON of ROR juvenile hatchery spring/summer Chinook salmon by their ROR travel times. The numbered data points correspond to the hypothetical samples in Figure 4A.14. X-axis is decreasing to correspond to seasonal pattern of travel times.

Discussion

In this study, a strong seasonal pattern in survival capacity of barged and ROR yearling Chinook salmon was detected even immediately after FCRPS passage. This supports other research studies that have found strong seasonal patterns with hypothesized selection processes in the estuary and ocean (Scheuerell and Williams 2005, Muir et al. 2006, Schreck et al. 2006, Eder et al. 2009a, Scheuerell et al. 2009, McMichael et al. 2010b). But this study is the first to detect a seasonal pattern of survival capacity before migration through post-FCRPS environments. Water temperature at time of collection at the barge loading site LGR/LMN and at BON after ROR passage (T_{site}) had the strongest influence on survival capacities of these fish and helped explain the strong seasonal patterns observed. Implicit in the T_{site} variable is the difference in temperatures that the fish experienced due the type of passage through the FCRPS. Water temperatures at a given time of the year are higher at LMN than at LGR and higher still at BON because the water warms up as it slowly passes through the reservoirs. Compared to ROR fish, LGR-barged fish experience cooler water temperatures up until the point of collection for barging and only experience the warmer water temperatures in the FCRPS for a possibly negligible amount of time that is on a scale of hours rather than days. Thus, the type of FCRPS passage affects T_{site} and indirectly affects survival capacity. Also, annual differences in survival capacity were detected, and it is possible that these were due to annual variation in environmental conditions or differences in handling during the study. Although more experiments were conducted in 2009 than 2008 (see Methods of Chapter 4), survival capacity was not lower in the second year of study. Thus, it is more likely that there were effects from annual differences in environmental conditions. Heterogeneity among individuals in their survival capacities was also detected. Interestingly, water temperature, and not the source of origin indices, was the most influential on heterogeneity among individuals. The low level of heterogeneity among the PIT-

tagged individuals may represent an effect from tagging or source of origin and ROR migration. By and large, water temperature had a strong influence on the survival capacity and heterogeneity of hatchery yearling Chinook salmon passing through the FCRPS.

The subset analysis with LGR-barged and LMN-barged fish supported the findings from the full analysis, and also provided insight into other possible factors by minimizing the effects from source of origin. These fish originated only from the Snake River and its tributaries with most of the fish originating from above LGR, while the full analysis included fish from the Upper, Middle, and Lower Columbia. In the subset analysis, water temperature still dominated among the explanatory variables, and thus reinforced the similar finding from the analyses that included ROR fish. In addition to water temperature, B_{LGR} and CF had some influence on survival capacity. Although T_{site} already incorporates some of the influences of barging on temperature experienced, the positive influence from the B_{LGR} index may be capturing other aspects of barging unrelated to water temperature. My results suggest that reducing the barging distance across the entire 461 km of the FCRPS by 106 km and instead of ROR migration between LGR and LMN reduced their survival capacities due to temperature-dependent and temperature-independent processes. The negative relationship between CF and survival capacity $\ln(m)$ could be due to increased metabolism of these fish during the higher water temperature challenges (Gillooly et al. 2001), or due to uptake of water (and weight) from stress (Wendelaar Bonga and Lock 1992, Anderson 2006). Overall, this subset analysis showed that even the difference in the type of passage experience across a section of the FCRPS elicits detectable effects. In the rest of the discussion, I will first compare the seasonal patterns observed in this study to those from other studies and consider the differences between barged and ROR fish survival capacity. I will then deliberate whether water temperature is truly the seasonal factor affecting the survival capacity

and heterogeneity of these fish. Finally, I will discuss my findings in context of survival capacities across life stages.

A seasonal change in the ratio of barge to ROR survival capacity m_B:m_{ROR} was detected. In other studies, the ratio of barged to ROR post-FCRPS fish survival (*D*, also termed differential delayed mortality) have been observed to begin below 1 early in the outmigration season, increase throughout the season, and sometimes decrease at the end of the season (Anderson et al. 2005, Muir et al. 2006, NOAA 2010, Anderson et al. 2011). The seasonal increase in *D* is thought to be, in some occasions, due to a seasonal decline in the SARs of ROR fish, while the SARs of the barged fish remained relatively constant, and in other occasions, due to a seasonal increase in survival rates of barged fish, while survival of ROR fish remained the same throughout the season. In some years, no seasonal pattern in the transport:migrant (*T*:*M* or *T*:*I)* ratio of SARs, which includes survival through the hydropower system, are detected based on the predictors used in the fitting models (Anderson et al. 2005, NOAA 2010). Furthermore, survival in the estuary has been observed to increase across the season for barged hatchery yearling Chinook salmon, and remain the same for their ROR counterparts (Eder et al. 2009a). Any differences in seasonal patterns of my observed survival capacities relative to the SARs from other studies may be because of the life stage at which they were estimated. The survival capacities from my study are in reference to Bonneville Dam immediately after passage through the FCRPS. Experiences accrued during migration down the Lower Columbia River and Estuary, in the ocean, and during upstream migration as adults will alter survival capacities of these fish. The absolute values of my m_{Barged}:m_{ROR} estimates cannot be compared to *D*, but interestingly the general seasonal patterns are similar between m_{Barged}:m_{ROR} and *D*. The values of m_{Barged}:m_{ROR} and *D* are likely not equivalent because different conditions and selection processes occur in the post-FCRPS environments that will modify the patterns of fish survival capacities. It is thus important to

consider the cumulative effects across life stages when examining SARs of Pacific salmon or other species with complex life cycles (Nickelson and Lawson 1998, COMPASS 2008, Crozier et al. 2008, Ferguson et al. 2008, ISAB 2008a).

A negative relationship between time spent in the FCRPS as ROR fish and survival capacity is suggested by my findings. Other studies have also detected greater health among barged fish relative to ROR fish. Arkoosh et al. (2006) tested a 10-day disease challenge by exposing barged and ROR fish to a lethal concentration of *Listonella anguillarum* known to yield 50% mortality (LC50). Barged fish had lower cumulative mortality than ROR fish in the 10-day challenge. Results from other studies on barging yearling Chinook salmon for only part of the FCRPS generally show support for the negative travel time vs. survival capacity relationship. In hatchery spring/summer Chinook salmon from 1994 to 2000, *D* decreased across the LGR (0.700), LGS (0.654), and LMN (0.502) transport sites (Williams et al. 2005). Transport from MCN appeared to be less advantageous to adult returns of barged fish in comparison to transport from LGR and LGS (Giorgi et al. 2002). In more recent research, transportation of Columbia River hatchery spring Chinook salmon from MCN yielded higher SARs than those of bypassed ROR fish, but not of undetected (i.e. spillway/turbine routes) ROR fish (Marsh et al. 2010b). The negative travel time vs. survival capacity relationship is also supported by my finding that decreasing FCRPS travel time of proxy ROR fish reduces the survival capacity loss from downstream passage. By the end of the season, when travel times were the shortest, the proxy ROR fish even gained survival capacity between LGR and BON. Based on my findings and other studies, barging is beneficial to the survival capacity of hatchery spring/summer Chinook salmon because it reduces time spent in the FCRPS.

Whether the patterns of survival capacity in this study are temperature-dependent (including direct and indirect effects) or are temperature-independent but influenced by other

seasonal factors is a central question to the effects of downstream passage experience on survival. Scheuerell et al. (2009) found that day of arrival below BON, at the estuary, was a better predictor than water temperature at BON for SARs. This suggests that a suite of seasonal processes more strongly affects SARs than water temperature alone. In my study, I found that T_{site} was the best predictor of survival capacity immediately after FCRPS passage compared to closely related factors including DOY of collection at Bonneville Dam (DOY_{BON}) (Appendix C, Figures A.3 and A.4). High correlation (0.82) occurred between T_{site} and DOY_{BON}. This suggests that immediately after FCRPS passage, water temperature has had a major influence on fish survival capacity, but that in post-FCRPS environments, many other processes are also involved. I now consider how water temperature can affect biological processes and in turn affect survival capacity.

In poikilotherms, energetic demands are closely related to environmental temperature because of its direct influence on biochemical reactions. Physiological optima in temperature have been determined in many species under various conditions (Brett 1971, Björnsson et al. 2001, Imsland et al. 2001). At temperatures higher than these optimum values, increased consumption sometimes cannot counteract the energetic demands at increased metabolic rates, thus resulting in reduced growth rates. Under limited food resources, fish prefer cooler waters to reduce their metabolic rate and required energetic intake (Despatie et al. 2001, Roessig et al. 2004). Crozier et al. (2010) observed increasing sizes of juvenile Chinook salmon as water temperatures increased from 10°C to 14°C at low population densities, but found the opposite pattern when population densities were high and resources were more limited. In my study, with the increase in temperature throughout the season, energetic demands increased, and this may be a reason for why the survival capacity of both barged and ROR yearling Chinook salmon was decreasing throughout the season. Temperatures during the downstream migration of the yearling

Chinook salmon in my study were between 7°C and 15.5°C with survival capacities being greatest at lower temperatures. This suggests that food resources were limited. If optimal growth was occurring at around 7°C under these limited resources, fish were growing at a maximum rate of 0.5% body weight per day (Brannon et al. 2004, developed from Brett et al. 1982). At this same level of food resources at 15°C, these fish were no longer growing and were only maintaining their weight. At temperatures greater than this, they were losing weight. In the FCRPS, ROR yearling Chinook salmon have been observed to have lower lipid and protein levels than barged fish (Congleton et al. 2005). Lipid reserves also decreased in ROR fish throughout the season. My results suggest that limited resources and temperature-influenced energetic demands were decreasing fish post-FCRPS survival capacities.

Significant reductions in preferred temperatures and critical thermal maximum temperatures have been observed to occur in fish that have experienced stressors such as hypoxia, toxicity, and changes in salinity (Stauffer 1986, Rutledge 1989, Welch et al. 1989, Monirian et al. 2010). Consistent with the seasonal patterns of temperature acclimation and survival capacity in my study, Sauter et al. (2001) observed a preference of lower water temperatures throughout the season in juvenile fall Chinook salmon from the Columbia River. Their preferred average temperature decreased from 16.7°C to 11.1°C as acclimation temperatures increased from 12°C to 18°C throughout the season (May 5 to August 7), with some occurrences of fish selecting water temperatures below 7.5°C. The authors thought the preference for cooler waters may be to preserve the ATPase activity in the gills during smoltification. Inhibition or reduced osmoregulatory response has been observed in steelhead at 13°C, in Coho and Chinook salmon at 15°C, and in Atlantic salmon at 16°C (Clarke et al. 1981, Zaugg 1981, Duston et al. 1991, from Sauter et al. 2001). Sauter et al. (2001) suggested that the preference for lower temperatures was for down-regulating physiological activities associated with

smoltification that include lipid depletion, increased hormones, morphological changes in the skin and scales, and developmental changes in the intestine, kidney and urinary bladder. The optimal osmoregulatory and metabolic performance occurs near 10°C in seawater (Clarke and Shelbourn 1985, Welch et al. 1995, from Sauter et al. 2001). In contrast, this preference for cooler waters over the season was not detected in juvenile spring Chinook salmon that had a preferred average temperature of 16.6°C throughout the season (Sauter et al. 2001). This may be due to the availability of food and the limited amount of stress they experienced during rearing at the hatchery and during experimentation at the laboratory. Eder et al. (2009b) observed a greater index of smoltification in ROR fish than barged fish, as well as an overall increasing pattern across the season among the fish. These patterns suggest that my fish were increasingly less capable of withstanding the increased water temperatures in my challenge experiments because of increased smoltification across the season as well as in ROR fish relative to barged fish. In an environment with many potential stressors, fish may also become increasingly susceptible to pathogens as water temperatures rise. Population growth rates of bacteria and fungi correlate positively with temperature (Roessig et al. 2004). As temperatures rise, the time to onset and the severity of diseases increases in some species, but not all (Kent 1992, Becker et al. 2006, Karvonen et al. 2010). The range of water temperatures permissive to pathogen viability, and the pathogen-host relationship across these temperatures are important (Beaman et al. 1999). Thus, dealing with disease as an additional stressor, may also explain the seasonal decline of survival capacity among barged and ROR fish in my study but it depends on the pathogen. Overall, the findings of other studies suggest that the seasonal decrease in survival capacity of the yearling Chinook salmon in my study may be caused by an increased need to cope with stress (caused by smoltification, disease, etc.) in order to regain or maintain homeostasis and health.

Mechanisms that are temperature-independent but still seasonal may also affect the survival capacity of hatchery yearling Chinook salmon after FCRPS passage. Early season migrating fish may be in better condition (i.e. higher survival capacity) than the rest of the group and thus ready to begin downstream migration. Later migrating fish perhaps were still growing and attempting to gain better condition such as increased growth and smoltification, but initiated their migration to the ocean before river conditions were too poor. The fish of poorest health were driven to migrate despite their need to still grow and gain health only to find that the conditions in the FCRPS were marginally better than pre-hydrosystem conditions. For this reason, the late-migrating proxy ROR fish may have gained survival capacity during FCRPS passage. Zabel and Williams (2002) suggested that there is a tradeoff between early migration (which generally yields high SARs) and delaying migration to grow (which would help increase SARs). Another temperature-independent but seasonal factor may be predation risk. Throughout the season, piscivorous fish likely grow larger, increase their gape size, and have greater energetic demands. Piscivorous birds such as Caspian terns and cormorants increase the proportion of salmon in their diet early in the season, but this decreases mid-way through the season (Bird Research Northwest 2011). Thus, piscivorous birds alone are likely not responsible for the seasonal decline in survival capacity of hatchery yearling Chinook salmon (Antolos et al. 2005), but may be interacting with other factors such as fish condition and swimming behavior (Schreck et al. 2006). Interactions between temperature-dependent (e.g. energetic demands, disease, smoltification, and swimming behavior) and temperature-independent factors (e.g. arrival timing and predation risk) may also co-occur.

It is important to note that this study only investigated the fish survival capacity referenced to Bonneville Dam immediately after FCRPS passage. I did not evaluate how migration through the Lower Columbia River, residence in the ocean, and migration of returning

adults affects a fish's survival capacities. For example, depending on when the fish arrive in the estuary, they may experience different levels of predation risk for piscivorous birds (Bird Research Northwest 2011) and marine fish (Emmett et al. 2006). Size-selective predation has been determined as a possible driver for differences between barged and ROR fish (Muir et al. 2006). My study also was not designed to test for the match/mismatch hypothesis in which juvenile salmon arrive at the ocean during the time upwelling occurs and there are abundant food resources (Cushing 1990, Pearcy 1992), density-dependent effects (Levin et al. 2001, Levin and Williams 2002, Buhle et al. 2009), and sufficient growth for the first year of survival in the ocean (Mahnken et al. 1982, Beamish and Mahnken 2001, Farley Jr. et al. 2007). During adult river migration, disruption of homing processes from poor imprinting during juvenile migration and poor health can occur (Keefer et al. 2008), but again is not tested in this study.

Overall, differences in the survival capacities immediately after FCRPS passage of LGR-barged, LMN-barged and ROR hatchery yearling Chinook salmon appear primarily related to the water temperatures they experienced. As water temperatures increased throughout the season, the decline in survival capacities among all of the tested fish, regardless of passage type, was likely strongly affected by increasing metabolic demands under limited resources. Disruption of homeostasis by stress could have further increased energetic demands, and thereby further decreased survival capacities. Also, my results show opposing forces of survival capacity: between the seasonal decrease in "overall" survival capacity which correlates with water temperature, and the decrease in survival capacity "loss" which relates to travel time. An influence from barging unrelated to temperature was also detected when comparing LGR- and LMN-barged fish. Temperature-independent but still seasonal factors of survival capacity include fish health prior to arrival at the FCRPS and timing of migration, as well as stress from predation risk. Although the exact mechanisms underlying the patterns of survival capacities have yet to be

elucidated, survival challenge tests were a convenient method to determine the relative benefit of the barge transportation mitigation strategy. Future studies could use this technique to determine differences in survival capacities at various life stages, post-FCRPS reaches, and dam passage histories that include the bypass route.

Subchapter 4B

Loss of Equilibrium in an Anesthetic Dose of MS-222: a Surrogate Endpoint to Estimate Post-Hydropower System Survival Capacity of Barged and ROR Fish

Abstract

I tested a simple and quick surrogate endpoint to compare to the survival capacity ln(m) of LGR-barged, LMN-barged, and run-of-river (ROR) hatchery yearling Chinook salmon that was examined in Subchapter 4A. The surrogate endpoint tested was loss of equilibrium (LOE) in an anesthetic dose of tricaine methanesulfonate (MS-222). MS-222 is a common anesthetic used on fishes during field research and surveys, and has the potential to be a quick assay for determining relative survival capacities of fishes. The time to LOE of all fish from the three different treatment groups declined over the season. This result suggests that metabolic rates increased as water temperature increased throughout the season. In contrast to the lower survival capacity of ROR fish relative to barged fish from Subchapter 4A, the time to LOE of ROR fish was greater than that of barged fish. A plausible reason for this opposite pattern is that ROR fish were experiencing limited food resources and lowered their metabolic rates to compensate for this. As a consequence, ROR fish were more slowly induced by MS-222 than barged fish. However, other studies have determined that juvenile salmon undergoing smoltification exhibit increased rates of metabolism, which is likely occurring at a greater rate in ROR fish than barged fish. Thus, more research would be needed to determine the appropriateness and usefulness of this assay.

Introduction

Surrogate endpoints are designed to eliminate the occurrence of mortality in studies, to minimize the pain animals experience, and to more quickly obtain results. The study that I conducted in this subchapter was designed with a surrogate endpoint to answer the same questions as the ones posed in Subchapter 4A: What is the seasonal pattern of barged and ROR survival capacities after FCRPS passage? What may be underlying mechanisms of these patterns? The surrogate endpoint I chose to test was time to loss of equilibrium (LOE) in an anesthetic dose of tricaine methanesulfonate (MS-222). MS-222 is often used as an anesthetic when handling fish for species identification, biological measurements, and tagging. The LOE in MS-222 is thus a behavior commonly observed, and if related to fish condition, could provide a useful technique as a surrogate endpoint.

MS-222 is generally absorbed through the gills, and can be absorbed through the skin of scaleless fish (see Carter et al. 2011 for a review). It is then distributed throughout the body by the blood circulatory system. MS-222 and the metabolized non-polar compounds are excreted through the gills, while the metabolized polar compounds are excreted through the kidneys. MS-222 is no longer detected in the blood after 8 hours or in the urine after 24 hours (Carter et al. 2011). The anesthetic action of MS-222 is primarily through a suppression of the nervous system (Burka et al. 1997): it binds to the Na^+ receptors of the Na^+/K^+ pump channels of a nerve, inhibiting it from repolarizing to its resting potential. Fish will first experience a light sedation with reduced swimming and slight loss of response to stimuli (Stage I), a light narcosis with loss of equilibrium but still some efforts to right the body (Stage II), a deep narcosis with complete loss of equilibrium (Stage III, level 1), a surgical level of anesthesia with total loss of reactivity and muscle tone (Stage III, level 2), and a medullary collapse with cessation of respiration and cardiac arrest that proceeds to death (Stage IV) (Burka et al. 1997). MS-222 is no longer detected

in the blood after 8 hours or in the urine after 24 hours (Carter et al. 2011). To which stages and how quickly fish experience the effects of MS-222 depend on the duration of immersion in the anesthetic solution, the dosage, and if they had been anesthetized recently.

In this study, I ran experiments to test for the LOE surrogate endpoint in an anesthetic dose of MS-222 in barged and ROR fish after passage through the FCRPS. These experiments were designed as a method to compare to the survival capacity measures observed in the challenge experiments of Subchapter 4A. If seasonal patterns in the times to LOE are detected, this may be a useful method in assessing fish condition, particularly when collected samples are already being anesthetized for fish processing.

Methods

Sample collection and experimentation

After collection of LGR-barged, LMN-barged, and ROR hatchery spring/summer Chinook salmon at Bonneville Dam in 2009 (Methods of Chapter 4), almost all of these fish (i.e. time-permitting) (see Table 4B.1 for sample sizes) were introduced to a challenge experiment to estimate overall fish condition. The time to LOE in an anesthetic solution of MS-222, at a concentration of 40 $mg \cdot L^{-1}$, was used as the surrogate endpoint. Prior to my experimentation, barged fish were last anesthetized at LGR or LMN before barge transportation, and ROR fish were last anesthetized at Bonneville Dam. LGR-barged salmon were allowed to acclimate for 1 to 3 days (on average 1.75 days), LMN-barged salmon 1 to 4 days (on average 2.5 days), and ROR salmon 1 to 3 days (on average 2.2 days) before the test trials. Because MS-222 was used to sample the transported fish prior to barging and to collect ROR fish at Bonneville Dam, the fish were acclimated to ensure no residual MS-222 was still present in the body during my challenge experiments. Batches of four to seven fish were immersed in a solution of MS-222 contained in a

white 19-liter plastic bucket. The fish were video-recorded for later viewing and accurate recordings of times to LOE. For each fish tested, I recorded the time to the first sign of LOE (Stage II response to MS-222; Burka et al. 1997), in which the side of their body was parallel to the bottom of the bucket. After the fish exhibited LOE, I measured the fish to the nearest mm and weighed them to the nearest 0.1 g. The solution of MS-222 was changed whenever the anesthetic solution appeared murky.

Data Analysis

The average times to LOE were calculated for each subsample. General multi-linear models of average times to LOE were analyzed to determine the 95% confidence set of best models, the AICc weighting of each explanatory variable, and the model averaging for each parameter (Burnham and Anderson 2002, Mazerolle 2011). The explanatory variables tested in the full model were water temperature at Bonneville Dam (T_{BON}; °C) because that was the water used to prepare MS-222 solutions, wet mass (M; grams), condition factor (CF; mg/mm^3×100), and treatment group (*Group*). One group (ROR2.09) was excluded because the time to LOE was considered an outlier (i.e. more than two standard deviations away from the mean ROR LOE times in the first half of the outmigration period). The best model from the model averaging analysis was also graphed.

Table 4B.1**.** Sample sizes of the three treatment groups of hatchery spring/summer Chinook salmon tested in the challenge of LOE in MS-222.

Treatment group	Sample name	Collection date at BON	N (collected)	n (challenged)
Run-of-River	ROR1.09	4/12/2009	83	83
	ROR2.09	4/19/2009	85	85
	ROR3.09	4/26/2009	84	84
	ROR4.09	5/1/2009	84	65
	ROR5.09	5/9/2009	84	50
	ROR6.09	5/16/2009	85	84 (videos lost)
	ROR7.09	5/23/2009	84	84
	ROR8.09	5/30/2009	81	81
	ROR9.09	6/5/2009	84	84
Barged from Lower Granite Dam	LGR-B1.09	4/10/2009	88	84
	LGR-B2.09	4/17/2009	82	82
	LGR-B3.09	4/24/2009	86	86
	LGR-B4.09	5/1/2009	83	83
	LGR-B5.09	5/10/2009	84	50
	LGR-B6.09	5/16/2009	84	59
	LGR-B7.09	5/23/2009	80	75
	LGR-B8.09	5/30/2009	85	85
Barged from Lower Monumental Dam	LMN-B5.09	5/10/2009	84	50
	LMN-B6.09	5/16/2009	84	83
	LMN-B7.09	5/23/2009	84	83
	LMN-B8.09	5/30/2009	46	45

Results

Treatment group (*Group*) and water temperature (T_{BON}) were the two explanatory variables present in all four models of the 95% confidence set (Table 4B.2 and Table 4B.3). Condition factor and mass were also part of the 95% confidence set, but the 80% confidence intervals of these parameters included zero. When testing the model with *Group* and T_{BON}, there was a statistically significant difference between the LGR-barged and ROR fish (p=0.034), but not between LMN-barged and ROR fish (p=0.147), nor between LGR-barged and LMN-barged fish (p=0.700) (Figure 4B.1). Similar to the results of survival capacity ln(*m*) from Subchapter 4A, there was a decline in the time to LOE as T_{BON} increased. In contrast to the results from Subchapter 4A and my current hypothesis, time to LOE was greater among ROR fish than barged fish.

Table 4B.2**.** General multi-linear modeling of average time to loss of equilibrium (LOE) in an anesthetic dose of MS-222: 95% confidence set with number of parameters (K), difference in bias-corrected AIC score from best model (ΔAICc), AICc weight across all models tested, and correlation coefficient (r^2). All models have an *a* intercept modified by *Group*. Explanatory variables are water temperature at Bonneville Dam at time of collection (T_{BON}), fish condition factor (*CF*), and fish wet mass (*M*). N=8 samples

Models of time to LOE in MS-222	K	ΔAICc	AICc Weight	r^2
T_{BON}	3	0.00	0.370	0.646
Group + T_{BON}	5	0.74	0.256	0.746
T_{BON} + *M*	4	2.50	0.106	0.660
Group + T_{BON} + *CF*	6	3.07	0.080	0.772
T_{BON} + *CF*	4	3.19	0.075	0.647
Group + T_{BON} + *CF* + *M*	7	3.77	0.056	0.820
Group + T_{BON} + *M*	6	4.48	0.039	0.754

Table 4B.3. General multi-linear modeling of average time to loss of equilibrium (LOE) in an anesthetic dose of MS-222: Explanatory variable weight by AICc, model-averaged parameter estimates (with unconditional standard errors), 80% confidence interval of parameters, and predicted direction of effect on hatchery spring/summer Chinook salmon survival capacity after passage through the FCRPS.

Variables (units) in Models of time to LOE in MS222		Parameter	Variable Weight Across 95% Confidence Set	Estimate (SE)	80% Confidence Interval	Effect on Survival Capacity
Intercept,	*Group* LGR-barged	$a_{LGR\text{-}barged}$		3.02 (0.74)	2.08, 3.97	
	Group LMN-barged	$a_{LMN\text{-}barged}$	1.000	3.04 (0.16)	2.84, 3.24	Positive
	Group ROR	a_{ROR}		3.32 (0.14)	3.14, 3.50	
T_{BON},	water temperature (°C)	b_{Tbon}	1.000	-0.12 (0.02)	-0.15, -0.09	Negative
CF,	condition factor [(mg/mm^3) x 100]	b_{CF}	0.209	1.10 (1.31)	-0.58, 2.77	Zero
M,	mass (g)	b_M	0.115	-0.005 (0.03)	-0.05, 0.03	Zero

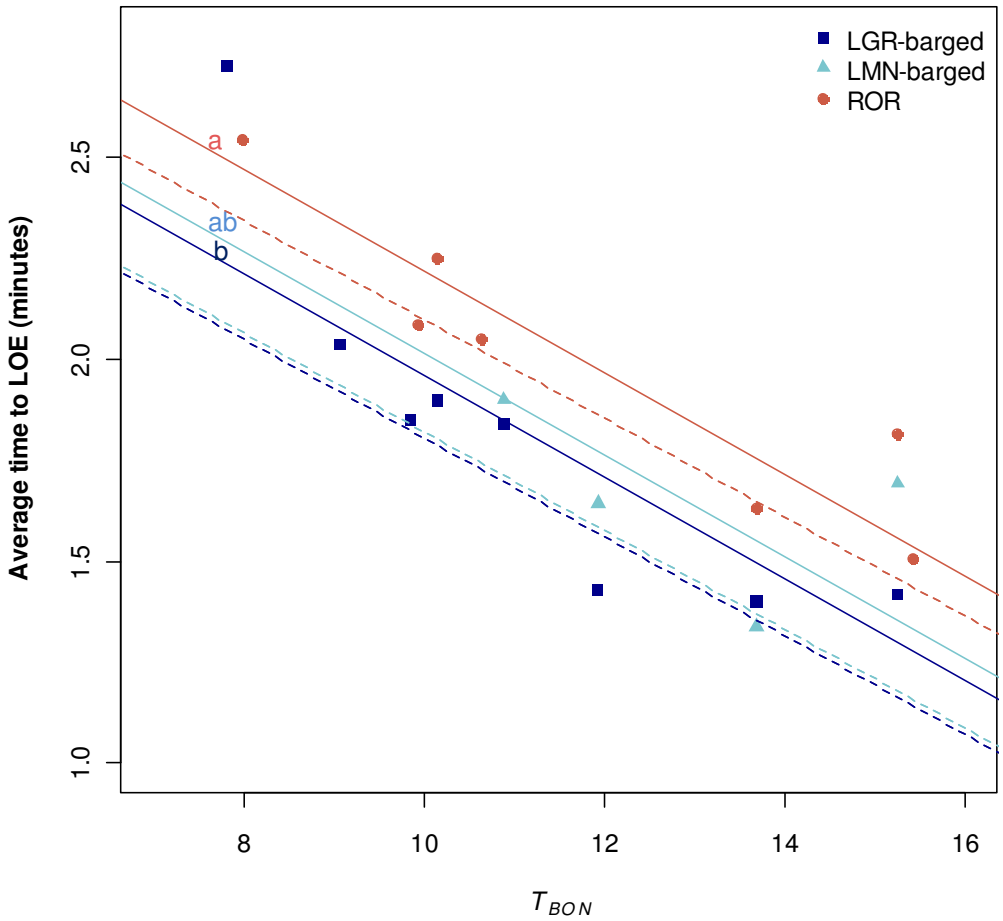

Figure 4B.1. Average time to loss of equilibrium (LOE) in an anesthetic dose of MS-222 of LGR-barged, LMN-barged, and run-of-river (ROR) fish after passage through the FCRPS. Data points represent observations. Dashed lines represent best model of the 95% confidence set with model-averaged parameters. Solid lines represent a general multi-linear model of average time to LOE by T_{BON} and *Group* (i.e. not model-averaged parameters). Different letters denote statistically significant differences between groups.

Discussion

The general seasonal decline in average time to loss of equilibrium (LOE), that is likely due to increased metabolic rates at higher temperatures (Paladino et al. 1980, Gillooly et al. 2001), is similar to the patterns of survival capacity observed in Subchapter 4A. However, the greater time to LOE in run-of-river (ROR) fish than LGR-barged fish was opposite to the lower survival capacity of ROR fish relative to barged fish (Subchapter 4A). ROR fish actually had

greater times to LOE than LGR-barged fish. The complexity of physiological processes and pharmacokinetics (i.e. how the body affects the potency and effectiveness of the drug) make accurately hypothesizing the patterns and relevant processes difficult. Two possible explanations for this pattern emerge from the literature. The first involves metabolic rates, and the second is related to the extracellular volume. Although both are unlikely occurring in the hatchery yearling Chinook salmon that I tested, future research may substantiate these hypothesized mechanisms, particularly the former.

Fish with high metabolic rates can quickly become induced by MS-222 and can also quickly remove MS-222 from their system (Burka et al. 1997). My results suggest that barged fish had higher metabolic rates than ROR fish. However, juvenile salmon undergoing smoltification exhibit greater metabolic rates (Seppänen et al. 2010, Björnsson et al. 2011). Congleton et al. (2003) observed barged wild and hatchery yearling Chinook salmon exhibited seasonal decreases in plasma triglycerides, increases in plasma lipase activity, and decreases in lipid content; all of which are consistent with increased metabolic rates. This pattern was also present in ROR yearling Chinook salmon (Congleton et al. 2005). Because ROR fish exhibit higher indices of smoltification than barged fish (Eder et al. 2009b), one would expect ROR fish to also exhibit greater metabolic rates than barged fish.

Many characteristics of fish condition can affect their metabolic rate. These include size, mass, stress, and disease status. Size-specific resting (post-absorptive) metabolic rates are generally lower in larger-sized organisms (Gillooly et al. 2001, Hunt von Herbing and White 2002). The relationship between time to LOE and mass was non-significant in my study. Thus, the mass-influenced metabolic rate was likely not important in determining the difference in condition between my barged and ROR fish. Stress, on the other hand, can come in various forms and can differentially affect metabolic rates. Environmental stress from limited food resources

can lower standard (post-fasting) metabolic rates (Djawdan et al. 1997). Thus, some stresses such as limited food resources could have lowered the metabolic rate of ROR fish and increased their time to LOE in my experiments. Disease can also increase or decrease routine (low activity) metabolic rates depending on the species of pathogen and host (Powell et al. 2005, Jones et al. 2007). Because of the possible variable responses from disease, barged fish could have increased metabolic rates and ROR fish could have decreased metabolic rates due to disease. High pathogen transmission rates can occur in fish barged at high densities (Dietrich et al. 2010). But disease challenges have shown barged fish to have greater survival capacity than ROR fish (Arkoosh et al. 2006), as well as greater survival in ROR fish than barged fish (Dietrich et al. 2011). Lower metabolic rates in ROR fish relative to barged fish can help explain why ROR fish exhibit longer times to LOE than barged fish. From the literature, the strong patterns of indices indicating increased metabolic rates in smolting fish make this hypothesized mechanism unlikely. But many factors such as limited food resources and disease can also affect metabolic rates. Thus, the patterns of metabolic rates in barged and ROR fish are still unclear.

Induction by MS-222 is negatively correlated with body mass (Houston et al. 1976, Burka et al. 1997). This relationship has been hypothesized to depend on both fish gill area and extracellular phase volume (Houston et al. 1976; Appendix D). In the first hypothesized process, uptake of MS-222 takes place primarily through passive diffusion through the gills (Hunn and Allen 1974). The gill surface area to body mass ratio has been hypothesized to affect the rate of induction by MS-222. The ratio between gill surface area and body volume declines as fish get larger. Thus, the effectiveness of MS-222 declines as fish get larger. In the second hypothesized process, larger fish have relatively less extracellular phase volume than smaller fish. Thus, the intracellular:extracellular ratio of MS-222 concentration may be greater in larger fish. With the greater concentration of MS-222 in the cell, MS-222 can more quickly get to the Na^+ binding

sites of the Na^+/K^+ pump and inhibit the nerve cell. MS-222 has to move into the cell before it can bind to the Na^+ binding sites of the Na^+/K^+ pump and inhibit the nerve cell from completing an action potential. MS-222 is lipid soluble and can easily pass through the lipid bilayer of the nerve cell membrane. The combination of these two processes determines the rate of MS-222 induction. The authors also noted that because these two processes are exponential, the rate of MS-222 induction is highly sensitive in smaller sized fish. In my study, the effect of mass on time to LOE was slightly negative, but non-significant. It is possible that the range of fish masses observed was not large enough to detect this effect on time to LOE. It is also possible that other effects related to metabolic rate obscured these effects of mass.

Although time to LOE in an anesthetic dose of MS-222 is a quick test with a simple surrogate endpoint, the different patterns observed between treatment groups can be difficult to interpret due to the complexity of metabolic processes and related pharmacokinetics. Future research would be needed to measure the standard metabolic rates of barged and ROR fish after hydropower system passage in order to support or refute the hypothesis that ROR fish have lower metabolic rates than barged fish. Furthermore, if metabolic rates indeed differ, measures of stress, disease, and food availability before and throughout the hydropower system environments will help better explain the patterns of time to LOE observed in this study.

Subchapter 4C

Fish Indices and Proximate Composition of Barged and ROR Fish After Passage Through a Hydropower System

Abstract

Different measures of fish conditions were estimated to help examine potential factors that affect the survival capacity of juvenile Chinook salmon after different types of passage through the Federal Columbia River Power System (FCRPS): barged from Lower Granite Dam (LGR), barged from Lower Monumental Dam (LMN), and migrated as run-of-river (ROR). These fish condition measures were body mass, fork length, condition factor, and proximate composition. The proximate composition examined included total body water, dry mass, total body protein, and total body fat, and were estimated from a biological impedance analysis (BIA). ROR fish weighed significantly more than LGR-barged fish and were greater in length than both groups of barged fish. Condition factor, which is an index of body mass standardized to length, significantly decreased throughout the season for LGR-barged and ROR fish. Issues with calibration of the BIA instrument and uncertainties in the appropriateness of the BIA models used to estimate proximate composition of the fish in this study lead to cautious interpretations of the results. Overall, small differences in proximate composition such as total body protein and total body fat are likely difficult to detect accurately in barged and ROR fish by BIA.

Introduction

Differences in the post-FCRPS survival of barged and ROR fish have been hypothesized to be caused by differences in fish condition such as length (Muir et al. 2006, Mesa et al. 2008), condition factor (Congleton et al. 2005, Dietrich et al. 2008), and protein and lipid reserves (Congleton et al. 2005). Biological impedance analysis (BIA) is a relatively new and promising technology that can non-lethally determine the mass of proximate composition in fish (Cox and Hartman 2005). This portable device can yield high correlations between predicted and observed masses of total body water (TBW), dry mass (DM), fat-free mass (FFM), total body protein (TBP), total body ash (TBA), and total body fat (TBF) with r^2 values greater than 0.9 and as high as 0.999 (Cox and Hartman 2005). Cox and Hartman (2005) tested their BIA models, that were developed and validated with brook trout, with multiple warm-water species of fish. They found high correlations with r^2 values greater than 0.85, and concluded that their models could be used in other species with bodies of similar geometrical shapes.

Measuring impedance refers to measuring the resistance (R) and reactance (Xc) of an electrical current passing through an organism's body (Cox and Hartman 2005). The resistance of a substance is the ratio between the voltage and the current that passes through it. More generally, resistance is a measure of how much electrical current is stopped by a substance. The reactance is the opposition of a substance to a change in voltage; and more generally, it is a measure of how much a substance can be a capacitor providing drag in a current. Cell membranes are formed of a non-conductive lipid bilayer, and thus currents at low voltages and high frequencies mostly pass through body components high in water such as extracellular fluid, blood, and muscle (i.e. cell membranes have high resistance). Peripheral, integral, and transmembrane proteins are also present in the lipid bilayer, and consequently the cell membranes can conduct electricity at higher frequencies (i.e. cell membranes have some capacitance). Thus, at moderate frequencies (50

kHz), observed measures of proximate composition can be regressed against measures of resistance and reactance to develop predictive models.

The objective of this study was to determine the mass, fork length, condition factor, and proximate composition (TBW, DM, FFM, TBP, TBA, and TBF) of LGR-barged, LMN-barged, and ROR fish using BIA models (Cox and Hartman 2005) in attempts to determine potential factors of their post-FCRPS survival capacities.

Methods

For each fish subsampled from my weekly collected treatment groups in 2009 (Figure 4C.1), I recorded their wet body mass to the nearest 0.1 g, their fork length (FL) to the nearest mm, and determined their condition factor [$(mg/mm^3)\times100$] from the body mass and FL measurements. For the BIA, I measured the distance between the electrodes (L) to the nearest mm, the resistance in series (R_m; ohm units) and the reactance in series (X_{cm}; ohm units) using the BIA instrument by RJL Systems, model Quantum X. I converted the R_m and X_{cm} measures into resistance in parallel (R_p; ohm units) and reactance in parallel (X_{cp}; ohm units) by the following equations:

$$R_p = R_m + (X_{cm}^2 / R_m)$$

$$X_{cp} = X_{cm} + (R_m^2 / X_{cm})$$

From the Cox and Hartman (2005) study that was conducted with juvenile brook trout (*Salvelinus fontinalis*), I estimated six measures of proximate composition in mass (g) using their following models:

Total body water: $TBW = 1.32045 + 3.46187(L^2/R_m)$

Dry mass: $DM = 0.29558\ DM + 4.31575\ (L^2/X_{cp})$

Fat-free mass: $FFM = -0.51881 + 0.99968(L^2/R_m)$

Total body protein: $TBP = -0.67352 + 0.89630(L^2/R_m)$

Total body ash: $TBA = 0.11516 + 0.11749(L^2/R_p)$

Total body fat: $TBF = -1.79087 + 1.80382^{-23}(L^2/X_{cp})$

The BIA instrument was occasionally tested for calibration throughout the field season. A subsample from each treatment group (total number of fish replicated = 110) was tested twice, once on the left side of the body and once on the right side. This helped determine how much error in measurements was performed, and the TBW model was used as a case example:

$$\text{difference in TBW replicates} = \frac{|TBW.replicate_1 - TBW.replicate_2|}{mean(TBW.replicate_1 + TBW.replicate_2)} \times 100\%$$

I also verified the appropriateness of these BIA models to the conditions of my study because differences in fish specimens and in the setup of the BIA instrument could bias my proximate composition estimates. I compared the predicted total mass (i.e. TBW + DM) to the observed mass of each fish to see if there was a 1:1 relationship.

I determined the proportions of each predicted proximate composition by total body mass which is a more appropriate way to compare the conditions of the fish. These were abbreviated respectively as TBW.P, DM.P, FFM.P, TBP.P, TBA.P, and TBF.P. To determine if there were any biases in these proportion estimates throughout the season and among treatment groups, I determined the proportion total body mass (i.e. TBW.P + DM.P, which should equal 1). In light of strong patterns of biases, I determined the proportions of each predicted proximate composition by the predicted total body mass, and interpreted the results with caution. Furthermore, because the TBF model from Cox and Hartman (2005) appeared to be incorrect (i.e. only yielded negative masses with values close to the intercept parameter because the slope was very small), I assumed the units should be micro-ohms instead of ohms. To determine an alternate estimate of fat content, I also estimated the percent fat in dry mass ($\%DM_F$) from percent dry body mass (%DM) in the following model determined by Hartman and Margraf (2008) with

juvenile Chinook salmon: $\%DM_F = -178.25 + 61.07 \times \ln(\%DM)$. For all predicted estimates, standard errors were determined by the delta method with 'deltamethod' function from the R statistical software package 'msm' (Jackson 2011).

Table 4C.1**.** Sample sizes of juvenile, hatchery, spring/summer Chinook Salmon collected at Bonneville Dam, weighed & measured, and tested for BIA.

Treatment group	Sample name	Collection date at BON	N (collected)	n (weighed & measured)	N (BIA-measured)
Run-of-River	ROR1.09	4/12/2009	83	83	35
	ROR2.09	4/19/2009	85	80	30
	ROR3.09	4/26/2009	84	84	33
	ROR4.09	5/1/2009	84	84	30
	ROR5.09	5/9/2009	84	84	31
	ROR6.09	5/16/2009	85	85	25
	ROR7.09	5/23/2009	84	84	25
	ROR8.09	5/30/2009	81	81	26
	ROR9.09	6/5/2009	84	84	25
Barged from Lower Granite Dam	LGR-B1.09	4/10/2009	88	88	33
	LGR-B2.09	4/17/2009	82	82	26
	LGR-B3.09	4/24/2009	86	86	31
	LGR-B4.09	5/1/2009	83	83	30
	LGR-B5.09	5/10/2009	84	84	25
	LGR-B6.09	5/16/2009	84	84	25
	LGR-B7.09	5/23/2009	80	80	26
	LGR-B8.09	5/30/2009	85	85	25
Barged from Lower Monumental Dam	LMN-B5.09	5/10/2009	84	73	25
	LMN-B6.09	5/16/2009	84	84	27
	LMN-B7.09	5/23/2009	84	84	25
	LMN-B8.09	5/30/2009	46	46	14

Results

The ROR fish weighed more than LGR-barged fish (p=0.004) but not LMN-barged fish (p=0.428), and were greater in FL than LGR-barged (p=0.004) and LMN-barged fish (p=0.17) (Figure 4C.1). On average, ROR fish were 3.7 g heavier and 6.0 mm longer than LGR-barged fish. No significant seasonal patterns were detected in the mass (p=0.055) or length (p=0.786) of the fish. The CF of LGR-barged fish and ROR fish decreased throughout the season (p=0.007). After accounting for the seasonal pattern in CF, no difference was detected between ROR and LGR-barged fish. However, LMN-barged fish had a significantly greater CF than LGR-barged

fish (p=0.004) and ROR fish (p=0.004). A number of LMN-barged fish appeared bloated. No statistically significant interactions between DOY of collection at BON and treatment group were found for mass, FL, or CF.

The predicted mass estimated from TBW and DM was correlated with the observed mass, and thus close to the 1:1 relationship (Figure 4C.2). The difference in replicate measurements of TBW was 6.02%. There was a strong seasonal pattern in predicted proportional mass which should equal approximately 1 at all times. The proportional mass increased significantly from below 0.7 to over 1.1 throughout the season ($p<0.001$; Figure 4C.3). There was no significant difference between the treatment groups.

When the proportions of proximate composition were standardized to the predicted masses (which helped alleviate the seasonal bias in the estimates), FFM.P and TBP.P showed no significant seasonal trends or any significant differences among groups (Figure 4C.4). A statistically significant seasonal decline in TBA.P was detected (p=0.041), along with greater TBA.P detected in LGR-barged than ROR fish (p=0.035). Although ROR fish had significantly greater TBF.P than LGR-barged fish (p=0.002), TBF.P estimates were negative and thus suspect. The $\%DM_F$ revealed no significant seasonal patterns or significant differences between the treatment groups (p=0.108; Figure 4C.5).

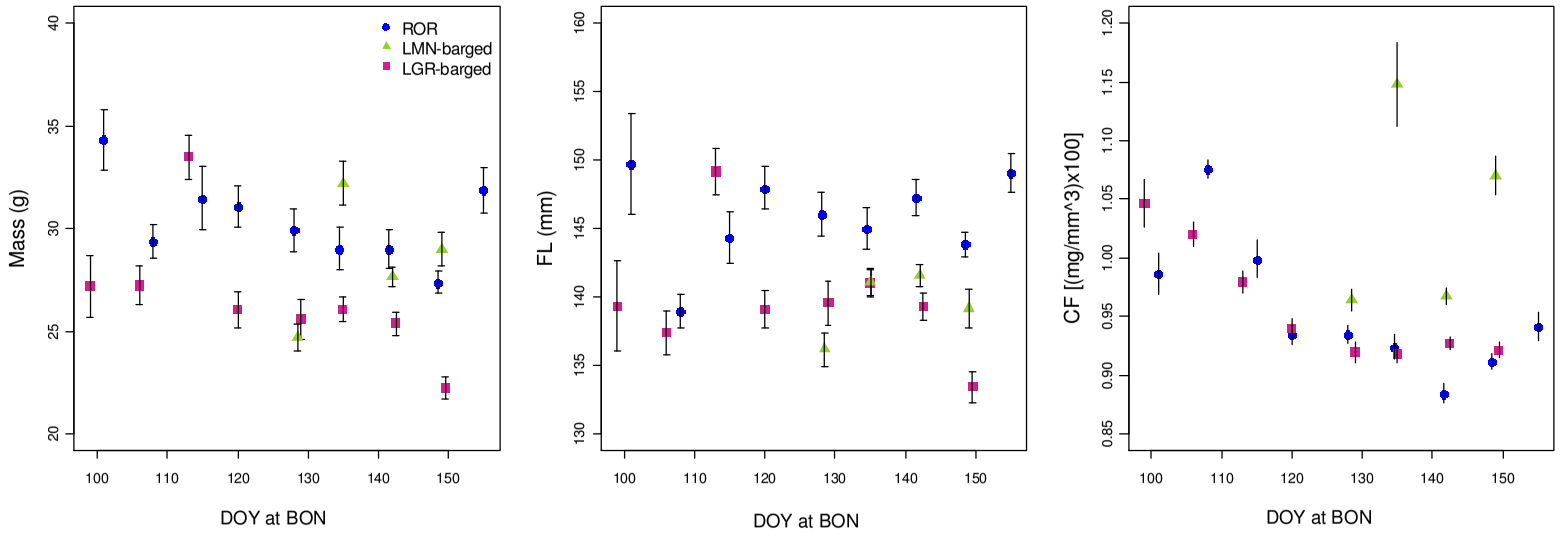

Figure 4C.1. Mass, fork length (FL), and condition factor (CF) of hatchery yearling Chinook salmon after different types of passage through the FCRPS (run-of river, ROR; LGR-barged; and LMN-barged). T-shaped error bars represent standard error determined directly from observed measurements; straight-lined error bars represent standard error approximated from the delta method (i.e. propagation of uncertainty).

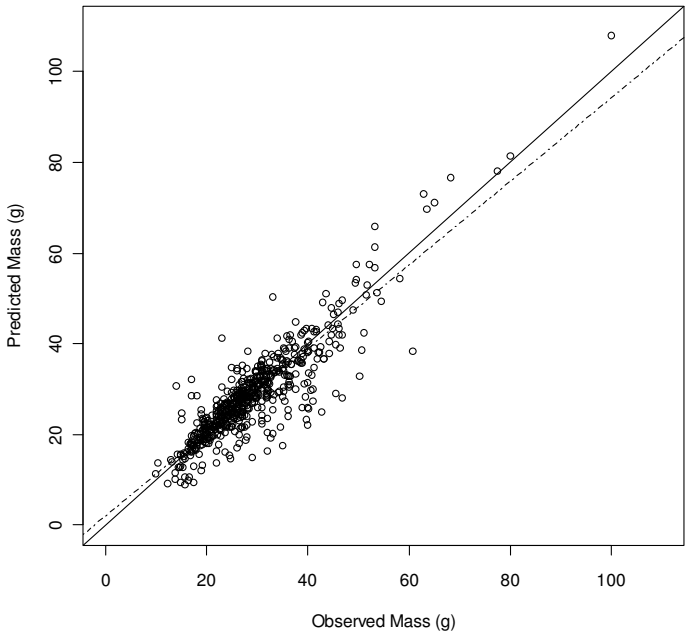

Figure 4C.2. Predicted mass from BIA-derived total water mass and total dry mass (models from Cox and Hartman 2005) by observed mass. N = 547, r^2 = 0.783. Solid line represents 1:1 line, and dashed line represents linear regression.

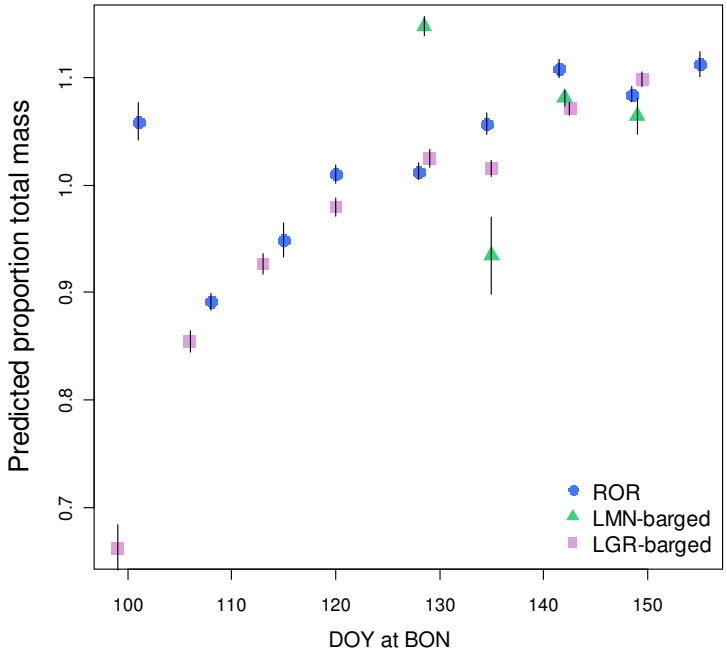

Figure 4C.3. Predicted mass from proportion TBW and proportion DM (i.e. should add to a proportion of 1). Error bars represent standard error estimated with the delta method.

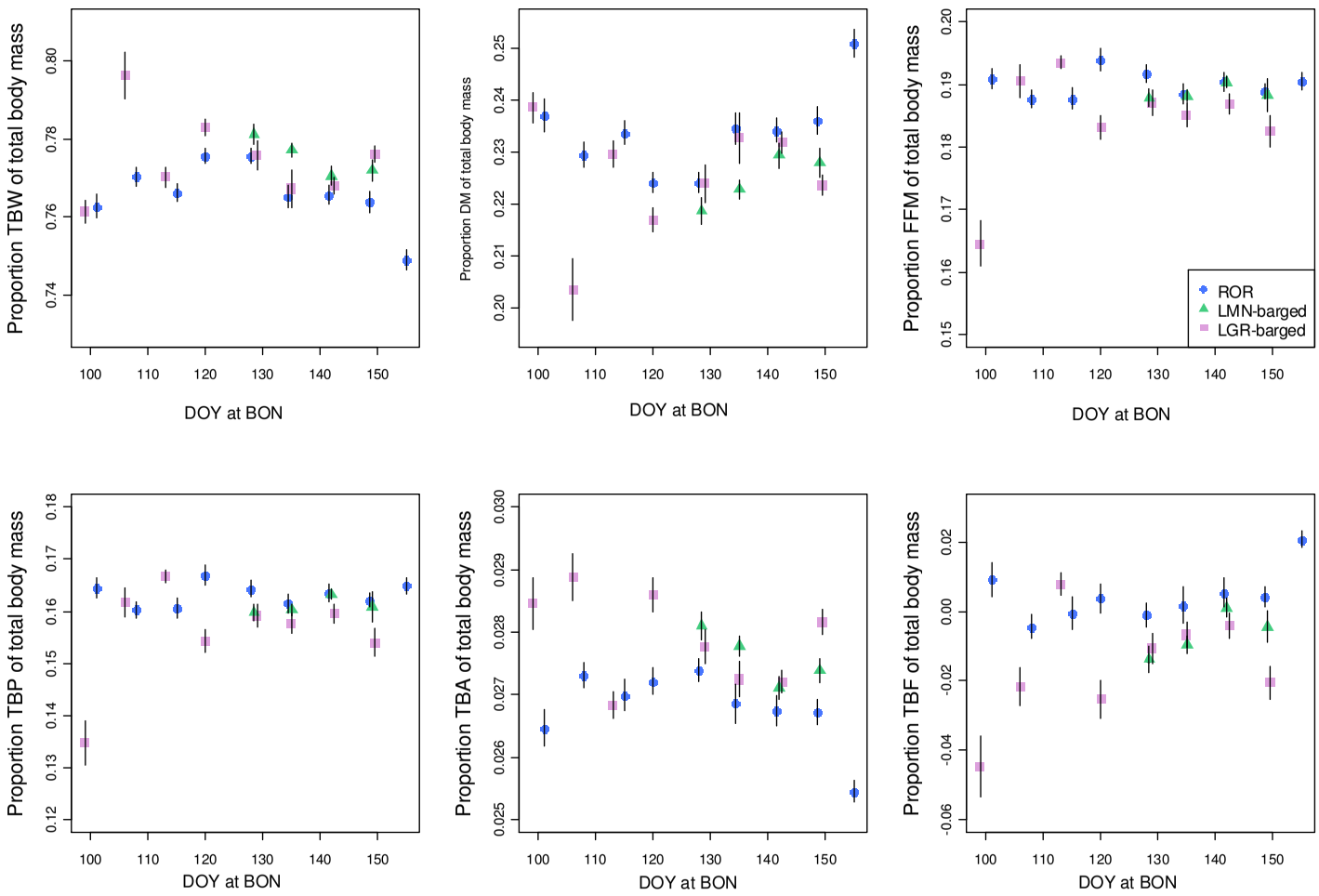

Figure 4C.4. Proximate composition by proportion of observed total mass of hatchery yearling Chinook salmon (run-of-river, ROR; LMN-barged; and LGR-barged) analyzed by biological impedance analysis (BIA). BIA models used were derived from brook trout (Cox and Hartman 2005). Error bars represent standard error approximated with the delta method (i.e. propagation of uncertainty).

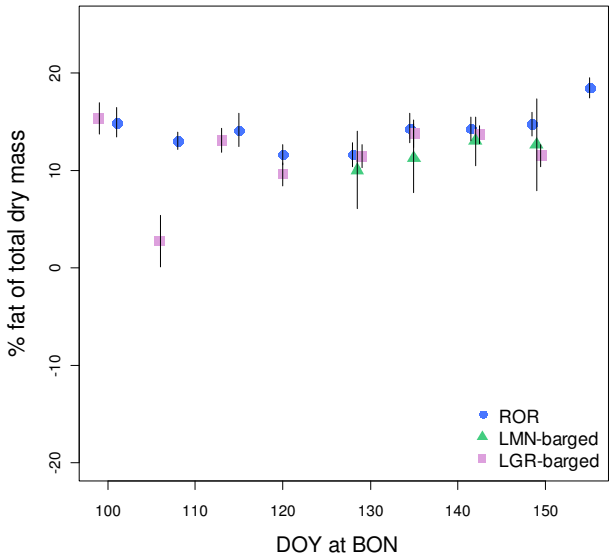

Figure 4C.5. Percent fat of total dry mass in barged and ROR fish at BON collected throughout the outmigration season of 2009. Estimates derived from model developed with juvenile Chinook salmon in Hartman and Margraf (2008).

Discussion

Run-of-river yearling Chinook salmon were larger in mass and in length compared to LGR-barged yearlings. This supports the findings of Muir et al. (2006) that ROR fish have the opportunity to grow while LGR-barged fish do not during their short passage through the FCRPS. The seasonal decline in CF among LGR-barged fish and ROR fish suggests that their metabolic rates were increasing with temperature and that food resources were depleting. Dietrich et al. (2008) also detected a seasonal decline in CF of ROR yearling Chinook salmon from Dworshak Hatchery but not Rapid River Hatchery. Interestingly, the CF determined in my study did not differ between LGR-barged and ROR fish. CF has been observed to be higher at LGR than at BON (Congleton et al. 2005, Dietrich et al. 2008). For example, the CF of hatchery yearling Chinook salmon averaged 0.95 at LGR and decreased to 0.85 at BON in 2003 (Congleton et al. 2005). These fish were in a negative energy balance in studies conducted from 2000 to 2003 with lipid and protein reserves decreasing from LGR to BON. My CF results do not support the hypothesis that hatchery LGR-barged fish experience a negligible loss of energetic reserves compared to hatchery ROR fish during FCRPS passage. This may be because my samples of ROR fish were run-at-large fish that included those originating from the Middle and Lower Columbia River. These fish may not have experienced as much depleted food resources as those that migrated from the Snake River. Another possibility is that food resources in 2009 were actually greater in the FCRPS than the pre-hydrosystem environment. The percentage of fish with empty stomachs has been observed to be lower at McNary Dam (3% in 1991) and Bonneville Dam (5% in 1991) than at Lower Granite Dam (26% to 38% from 1987 to 1991) (Muir and Coley 1996). Condition indices can sometimes be good predictors of proximate composition (Kaufman et al. 2007), but at other times assuming that CF and lipid content are correlated can be erroneous (Trudel et al. 2005, Peters et al. 2007) – especially if stressed fish are experiencing osmotic

imbalances and taking in water mass which is void of energy. This may have been the case for some LMN-barged fish in my study. For this reason, I attempted to gain more information on proximate composition.

Unfortunately, the proximate composition estimates were inaccurate because models from an unsuitable substitute were used, and validation with the specimens in this study was not performed. In general, the volume of a substance can be determined with a relationship between the distance between the electrodes, the impedance of the substance, and the specific resistance of a substance. However, these estimates can vary depending on specific conditions of the organism, the population, and the environment (Kyle et al. 2004, Barbosa-Silva and Barros 2005). A large body of research on BIA is available in the medical field where the technology was first utilized and where many influences on the accuracy of BIA measurements have been investigated. An inverse relationship exists between impedance and temperature (Gudivaka et al. 1996, Kushner et al. 1996, Cornish et al. 1998). Thus, any declines in my impedance measurements due to increasing temperatures across the season would bias my estimates of proximate composition. The level of hydration can also affect the conductivity and impedance measures and bias fat estimates (O'Brien et al. 2002, Barbosa-Silva and Barros 2005). Stress-induced imbalances in osmoregulation could affect the BIA estimates in my fish. One assumption of BIA modeling is that the substance measured has a volume of fluid of normal hydration status (Buchholz et al. 2004). BIA is conducted with a specific electrical frequency (50 kHz) that produces an impedance used to measure both extracellular and intracellular fluids (i.e. an impedance that is a mix of resistance and reactance). TBW can accurately be predicted with the 50 kHz resistance only because of its high correlation with extracellular water (ECW) in “normally” hydrated individuals. Deviations from the “normal” ECW:ICW compartmentalization can occur during starvation and disease. Also, using a non-conductive surface is also important (Lukaski 1996).

Moisture on the fish and on the processing table could alter the BIA measurements in this study. Thus, the advantages of this non-lethal, quick, and relatively inexpensive technique is advantageous only if the models have been validated by the collected samples, or appropriate substitutes, processed with techniques of "gold standard", and if there is strict adherence to the developed protocol which is often difficult in the field (Kushner et al. 1996, Cornish et al. 1998, Kyle et al. 2004, Barbosa-Silva and Barros 2005). The strengths in the relationships between indices and proximate composition can vary among populations, and thus population-specific validation may be necessary (Kaufman et al. 2007).

In addition to the issue of accurate proximate composition estimates, there is the issue of data precision when attempting to detect changes in small measurements of fish condition. ROR yearling Chinook salmon migrating from LGR to BON decreased significantly in lipid content from 1.75% by percent body mass to 0.64% in 2003 (Congleton et al. 2005). Similarly in 2007, lipid content showed a non-significant trend of lipid decrease in ROR fish from LGR (2.6%, Dworshak; 1.7%, Rapid River) to BON (0.5%, Dworshak; 0.4%, Rapid River) (Dietrich et al. 2008). These levels of lipid content were > 90% lower than the levels recorded at the hatcheries prior to release (Congleton et al. 2005, Dietrich et al. 2008). Protein content decreased from15.4% at the hatchery, to 14.5% at LGR, and then to 13.8% at BON (Congleton et al. 2005). Furthermore, the variability in my replicate measurements appeared to be high and could be due to handling, or differences between the left and right side proximate compositions. The BIA models of Cox and Hartman (2005) had high correlations perhaps because the fish spanned a large range of body sizes. Hatchery yearling Chinook salmon at Bonneville Dam are generally in the minimum level of lipid content that was tested in Cox and Hartman (2005). Upon visual inspection of the error in the data points in this lower range, there appears to be quite a bit of variability. Variation in these proximate composition estimates due to differences among stocks,

individuals, and biological and environmental processes may not be captured in the BIA model can cause difficulties in detecting differences in lipid and protein content between barged and ROR fish. This leads to the question of whether these small decreases in lipid and protein content are biologically significant and have an effect on survival. One could argue that these small differences in energy reserves at nearly depleted levels are important overwinter (Biro et al. 2004), and in their first year in the ocean (Beamish and Mahnken 2001).

Other studies have consistently observed a negative energy balance when comparing hatchery yearling Chinook salmon at BON to those at LGR. LGR-barged fish collected at BON are assumed to be the same as ROR fish collected at LGR. Hatchery yearling Chinook salmon decreased in lipid reserves during ROR migration from LGR to BON in each year of study from 1999 to 2003 (Congleton et al. 2005). The fish decreased in protein reserves during their ROR migration through the FCRPS with changes equivalent to –14%, –21%, –19%, and –15%, respectively for years 2000 to 2003. Nutritional indices (plasma protein, cholesterol, and alkaline phosphatase) decreased as fish migrated from LGR to BON. Thus, either my data were inaccurate, or my data actually were accurate and annual variability may be the reason why my year of study was different from the years examined in other studies. One study with samples collected at McNary Dam and Bonneville Dam in 1991 support the hypothesis of higher food resources in the FCRPS than in the pre-hydrosystem environment (Muir and Coley 1996). This would support my result of greater lipid content in ROR fish than barged fish. However, the lower survival capacities of ROR fish (Subchapter 4A) and hypothesized lower metabolic rates due to decreased food resources in the FCRPS (Subchapter 4B) do not support my possibly biased results on proportion total body fat. Also, there was no significant difference in my $\%DM_F$ results among the three types of FCRPS passage. But, these were also based on BIA-derived DM estimates. If one were also to believe the estimates of total body ash, the data suggests there was a

seasonal decline in the non-aqueous residue consisting mainly of salt and inorganic constituents such as Na^+, K^+, and Ca^{2+}. There was also more total body ash in LGR-barged fish than ROR fish. These patterns may reflect an overall decline in nutrition over the season and lower nutrition in ROR fish compared to LGR-barged fish. Overall, my BIA-derived estimates of proximate composition should be interpreted with great caution.

Several improvements on the accuracy and precision of BIA-derived proximate composition estimates have been suggested. Log-transformations could help improve model fits (Kotler et al. 1996). Tissue-specific BIA modeling could improve estimates of body components that are more locally distributed in the body. Fat in fish is more ventrally located but the BIA electrodes are generally placed in a more dorsal location, thus possibly underestimating the amount of fat in the fish (Cox and Hartman 2005, Pothoven et al. 2008). Multi-frequency BIA has been found to be more precise than single frequency BIA (Cornish et al. 1998; Demura et al. 2004). Also, phase angle and bioelectrical impedance vector analysis have been recommended as better methods of estimating proximate composition in practice (Barbosa-Silva and Barros 2005). Based on my study, I recommend deriving new proximate composition models specific to the population of fish examined, validation of the models with collected fish samples, and if accuracy and precision are still problematic, to implement one of the recommendations mentioned above, depending on the research question and budget at hand.

Summary and Conclusions

1. *Hydropower system passage type*: Juvenile, hatchery, spring/summer Chinook salmon that passed the Federal Columbia River Power System (FCRPS) as run-of-river (ROR) fish, barged fish from Lower Granite Dam (LGR), and barged fish from Lower Monumental Dam (LMN) each exhibited different survival capacities once they arrived at Bonneville Dam (BON), the downstream end of the hydropower system. My data from the survival challenge experiments and the exploratory analysis of proxy ROR fish suggests that minimizing the time spent in the FCRPS as ROR fish benefits their survival capacities.
2. *Seasonal pattern and water temperature*: There was also a strong seasonal decline in survival capacities across all three treatment groups, an effect stronger than that from the different types of FCRPS passage. The water temperatures that the yearling Chinook salmon recently experienced prior to their arrival at BON depended on their type of FCPRS passage and was the strong driver of this seasonal decline in survival capacities.
3. *Energetic demands and metabolic rates*: Throughout the season, the decline in survival capacities and increase in water temperatures suggest that energetic demands of the fish increased and food resources were limited. This is supported by the decline in condition factor observed in LGR-barged fish and ROR fish. Results from the challenge test with the surrogate endpoint of loss of equilibrium (LOE) in an anesthetic dose of tricaine methanesulfonate (MS-222) suggest that ROR fish had lower metabolic rates than barged fish to compensate for low food resources.
4. *Proximate composition*: The biological impedance analysis (BIA) yielded results on proximate compositions of my fish that were inaccurate. Population- and context-specific

validation of the BIA models as well as strict adherence to BIA protocols, which is sometimes difficult in the field, would have improved the estimates.

5. *My results in the context of differential delayed mortality*: Before the hatchery yearling Chinook salmon entered the post-hydrosystem environments, I was able to detect differences in fish survival capacities by their different types of FCRPS passage. It is important to note that the post-FCRPS smolt-to-adult return rates depend on the fish's cumulative experiences and natural selection processes in the Lower Columbia River and Estuary, the ocean, and during upstream migration. Even so, my results support the findings of other studies that have investigated post-FCRPS survivals in that seasonal effects can be stronger than the effects from different FCRPS passage type.

Chapter 5

Distributions and Critical Thresholds of Fish Condition Indices in Coho Salmon Under Lethal Stress

Abstract

Because dead or dying fish are rarely collected in the wild, it creates a challenge to determine to what degree a population is experiencing lethal stress. To provide insight into the possible relationship between mortality and stress at the level of the individual and the population, I experimentally challenged hatchery juvenile Coho salmon (*Oncorhynchus kisutch*) to increased water temperature, in the absence of food, to examine how the distributions of fish condition indices evolved as mortality rates increased, and to determine critical thresholds at the time of mortality or loss of equilibrium. The fish condition indices observed were fork length, wet mass, dry mass, proportion water mass, wet condition factor (CF), and dry CF. The distributions of "Live" fish condition indices were examined in five subsamples which occurred on challenge days (and respective mortality rates; "Mortality" fish) 0 (0%), 7 (0%), 12 (22%), 14 (40%), and 20 (60%). The critical thresholds were based on the "Mortality" fish collected at least three times a day throughout the challenge. Significant differences between the "Live" and "Mortality" fish in their relationships between fish condition index and challenge day were detected in all fish condition indices except for wet CF. The "Live" fish were progressively deteriorating in condition as indicated by the mode and the shape parameter of an Inverse Gaussian distribution of all six fish condition indices. The critical thresholds observed suggest that the "Mortality" fish were increasingly more tolerant of the sustained challenge conditions, and reasons for this pattern are discussed. L-skewness significantly increased for dry CF and non-significantly trended in the same manner for fork length, wet mass, and dry mass. Overall, my study provides weak support, but not rejection, of L-skewness of dry CF as an index to detect a fish population under lethal stress.

Introduction

Determining the degree of stress a population experiences is desirable to scientists, managers, and stakeholders. Some broad stresses that fish encounter are increased water temperatures, pollution, and limited food resources. However, determining the level of stress in a population and how it affects mortality is often difficult to obtain, especially when one cannot collect naturally dying organisms. Also, long-term baseline data are often not available to determine whether individuals are in optimal or poor condition.

Under poor environmental conditions, individuals progress towards a pathophysiological state in which energetic demands exceed supply. Mortality occurs when individuals reach a critical threshold. McEwen and Wingfield (2003) termed the state in which organisms have a negative energetic balance that is advancing towards this critical threshold, "allostatic overload". One example of a population that has dramatically decreased in numbers possibly due to poor conditions of individuals includes the population collapse of the Atlantic cod (*Gadus morhua*) in the 1990s (Lambert and Dutil 1997b, Dutil and Lambert 2000). Condition factor (CF = somatic weight in grams / (fork length in centimeters)^3 ×100) was an index that showed clear distinction between the control group of cod (0.83-0.98) and starved (0.42-0.67) or deceased cod (0.36-0.56). The ranges of CF for the starved cod overlapped with that of the deceased cod, but neither of these overlapped with the range for the control group. Decreases in fish's conditions can also occur at smaller time scales. Salmon that are migrating from the ocean to their spawning grounds cease feeding and swim against the current, sometimes for hundreds of kilometers in distance and hundreds of meters in elevation (Crossin et al. 2004). Migrating and spawning sockeye salmon died at the critical threshold of 4 MJ·kg^{-1}. However, baseline data are sometimes not available and snapshot data, which are more easily attainable, may provide valuable information if one knows

the shape of the probability density distributions of condition indices. Establishing critical thresholds would provide additional information to compare to these distributions.

A condition index is generally expected to be normally distributed for a healthy population that is not under selection (Anderson 2000, Anderson et al. 2008). As the conditions of individuals deteriorate, the shape of the distribution is expected to become skewed. Individuals with conditions near the critical threshold are expected to die which results in a one-sided truncation of the distribution, and this has been demonstrated in simulation modeling (Finstad et al. 2004). In one study conducted from 1997 to 2001, adult sockeye salmon entering the Fraser River had normally distributed levels of energy density (Rand et al. 2006) (or more generally, energetic reserves). Once they reached the spawning grounds, there was evidence of a left-truncated distribution in years of relatively poor migratory conditions. Also, the average energy density in the first quartile of the distribution decreased as sockeye salmon migrated upstream towards the spawning grounds and then leveled off at the critical threshold near and at the spawning grounds. Changes in the frequency distribution of trait characteristics have also been hypothesized to occur during the processes of natural selection (Wade and Kalisz 1990, Hackney and Durako 2004, Reizopoulou and Nicolaidou 2007).

A plethora of fish condition indices have been developed over the last century or so. These generally fall into three categories of bioenergetics, physiological stress, and genetics. Some examples include CF, energy density, plasma concentrations of cortisol, lactic acid, antioxidants, RNA/DNA ratios, and somatic indices such as percent mass in lipids and protein (Buckley 1984, Nash et al. 2006). Among these indices, CF is one of the easiest and quickest to measure. However, stress can disrupt the osmoregulatory balance in fish and cause increased water permeability (Bonga and Lock 1992, Van Pelt et al. 1997, Anderson 2006), thereby biasing estimates of CF and overestimating a fish's health condition. Although percent water mass which

is void of biological energy has not been directly related to mortality, reduced osmoregulatory ability detected in blood analytes has been used to predict mortality (Donaldson et al. 2010, Jeffries et al. 2011). Furthermore, a CF estimated with dry mass may provide a more accurate fish condition index because energy density correlates to percent dry mass of the whole body (Hartman and Brandt 1995, Pedersen and Hislop 2001), liver and muscle (Lambert and Dutil 1997a). Additionally, a fish condition index standardized to body length (i.e. dry CF) may be more appropriate than one standardized to mass (i.e. percent dry mass), particularly in fish with disrupted osmoregulation. Thus, dry CF is hypothesized to be a better fish condition index than proportion water mass and wet CF.

The main goals of this study were to first, determine critical thresholds of a several simple fish condition indices and then, explore the change in distributions of fish condition indices as the challenge experiment progressed. Additionally, I examined whether a CF estimated from dry mass provided more accurate measures of a fish's condition than one from wet mass. This study was conducted with juvenile Coho salmon introduced to a challenge experiment of increased water temperature without food.

Methods

Experimentation

To examine fish condition indices of Coho salmon under stress and artificial selection, a challenge of increased water temperature and starvation was performed. The Coho salmon were strains from two hatcheries (Issaquah Washington Department of Fish and Wildlife and University of Washington hatcheries), and were approximately 1.5 months old. Six subsamples of 151 fish each were placed in six 114-liter tanks. To begin the challenge, the water temperature was gradually increased over 3 days from that at which they were raised (8°C) to the target

challenge temperature (21°C). All fish were observed for mortality and loss of equilibrium (LOE) at least three times a day. If loss of equilibrium was observed, these fish were euthanized with tricaine methanesulfonate (MS-222; 250 mg/L buffered to pH 7.0 with $NaHCO_3$). The dead and LOE fish were categorized as the "Mortality" fish. The "Live" fish used to compare against the "Mortality" fish consisted of subsamples euthanized at specified times during the challenge. Subsample 1 was euthanized at the onset of the challenge and considered the control group. Subsample 2 was euthanized 7 days into the challenge. Subsamples 3 to 5 were euthanized once 22%, 40%, and 60% of the fish in each of their respective subsamples had died or showed signs of LOE. One of the six subsamples was excluded from further analysis because it had a rapid mortality rate of 83% by the eleventh day of the challenge. All of the "Mortality" and "Live" fish were processed for fork length (mm), wet mass (g), dry mass (g), proportion water mass, wet condition factor [wet CF = (wet mass in g) / (length in cm)^3 × 100], and dry condition factor [dry CF = (dry mass in g) / (length in cm)^3 × 1000]. Fish were dried in an oven at 60°C until the mass remained unchanged for at least two days.

Data Analysis

I first compared the fish condition indices between the "Mortality" and the "Live" fish throughout the challenge. Linear regressions were performed on each of the six fish condition indices against the time at which they were sampled during the challenge. For each fish condition index, one regression was performed for the "Mortality" fish and one for the "Live" fish. The linear regressions for the "Live" and the "Mortality" fish were compared by an analysis of co-variance (ANCOVA) with challenge day as the covariate. The condition indices of the "Live" fish for each of the subsamples were also fitted by the probability density function of an Inverse Gaussian (IG) distribution (Eq. 1). This helped to visualize how the distributions of each fish

index evolved throughout challenge with respect to the 25th percentile and median of the fish condition indices observed in the "Mortality" fish. To quantify the evolving pattern of these measurements, three different types of statistical measures were each regressed against challenge day. These statistical measures were the mode, the shape parameter of the IG distribution (λ; Eq. 2), and the L-moment skewness (also termed L-skewness; t_3) from an unknown distribution (Hosking 1990, Karvanen 2011). These three statistical measures were chosen for different reasons. The mode is a simple statistical measure that could detect the deteriorating conditions of the population. The λ parameter provided information about the shape of the distribution of the population by incorporating the mean and the variance. I used L-skewness as a statistical measure hypothesized to indicate that individuals in a population are dying and that there is a selection process on the weakest individuals (Anderson 1992, Anderson 2000, Rand et al. 2006). Also, L-moments are similar to the conventional moments, but differ in that they are "expectations of linear combinations of order statistics" (Hosking 1990). L-moments have been preferred over conventional moments because they are less influenced by single or a small number of outliers in small datasets, thus producing more robust inferences about the underlying probability function distribution (Hosking 1992). A test statistic (Z_{t3}) adapted from Cramer (1997) was used to determine whether L-skewness was statistically significantly different from zero (Eq. 3). Statistical significant skewness at approximately $\alpha = 0.05$ occurs with $-2 < Z_{t3} > 2$; but no conclusion can be reached about whether the distribution is skewed or symmetric with $-2 > Z_{t3} < 2$.

$$f(x;\mu,\lambda) = \left[\frac{\lambda}{2\pi x^3}\right]^{\frac{1}{2}} \exp\frac{-\lambda(x-\mu)^2}{2\mu^2 x} \qquad \text{(Eq. 1)}$$

Where x is the dataset, μ is the mean, and λ is the shape parameter of the IG distribution.

$$\lambda = \frac{\mu^3}{\sigma^2} \quad \text{(Eq. 2)}$$

Where μ is the mean and σ^2 is the variance.

$$Z = t_3 / SES \quad \text{(Eq. 3)}$$

Where the standard error of skewness (SES) is:

$$SES = \sqrt{\frac{6n(n-1)}{(n-2)(n+1)(n+3)}} \quad \text{(Eq. 4)}$$

And where n is the sample size.

Results

The "Live" juvenile Coho salmon showed progressively deteriorating conditions based on the decreasing patterns of fork length, wet mass, dry mass, wet CF and dry CF and increasing pattern of proportion water mass throughout the challenge from day 0 (subsample 1; control) to day 20 (subsamples 2 to 5) (Figure 5.1). "Mortality" fish showed a constant critical threshold for wet mass and dry mass. The other indices showed a moving critical threshold throughout the challenge of progressively worse condition at time of death or loss of equilibrium (Figure 5.1), except for fork length. Fork length of "Mortality" fish increased throughout the challenge. The linear relationships between each fish condition index and challenge day were significantly different between the "Live" and "Mortality" fish ($p<0.001$), except for wet CF (ANCOVA; $p = 0.170$).

In a visual comparison of the fitted Inverse Gaussian distributions of the "Live" fish condition indices to the mean of the "Mortality" fish condition indices (Figure 5.2), a constant critical threshold was most apparent for wet mass, dry mass, and percent water mass. Please note that only subsamples 3 to 5 had fish mortalities during the challenge experiment, and were

hypothesized to show truncations of the distribution at a critical threshold. For wet mass, fork length, and proportion water mass, the left tails of the distributions for subsamples 3 to 5 were near the 25th percentile of each respective "Mortality" fish condition index. Please see Appendix E, for the frequency distributions and fitted inverse Gaussian distributions for each subsample of each fish condition index.

Among the three statistical measures tested for patterns of progressively deteriorating fish condition, only dry CF consistently showed significant patterns. The mode of fish condition indices over the course of the challenge significantly declined for dry mass ($p = 0.018$), wet CF (0.031), and dry CF (0.009), and increased for proportion water mass ($p = 0.004$) (Figure 5.3). The shape parameter of the IG distribution significantly decreased over the course of the challenge for wet mass ($p = 0.043$), dry mass ($p = 0.047$), wet CF ($p = 0.009$), and dry CF ($p = 0.026$), and increased for proportion water mass ($p = 0.043$) (Figure 5.4). All estimates of L-skewness had $-2 > Z_{t3} < 2$, thus I failed to reject the statistical null hypothesis that the subsamples examined were symmetric. Nonetheless, dry CF significantly increased in L-skewness over the course of the challenge ($p = 0.047$) (Figure 5.5). Fork length, wet mass and dry mass showed a non-significant trend of increasing L-skewness throughout the challenge.

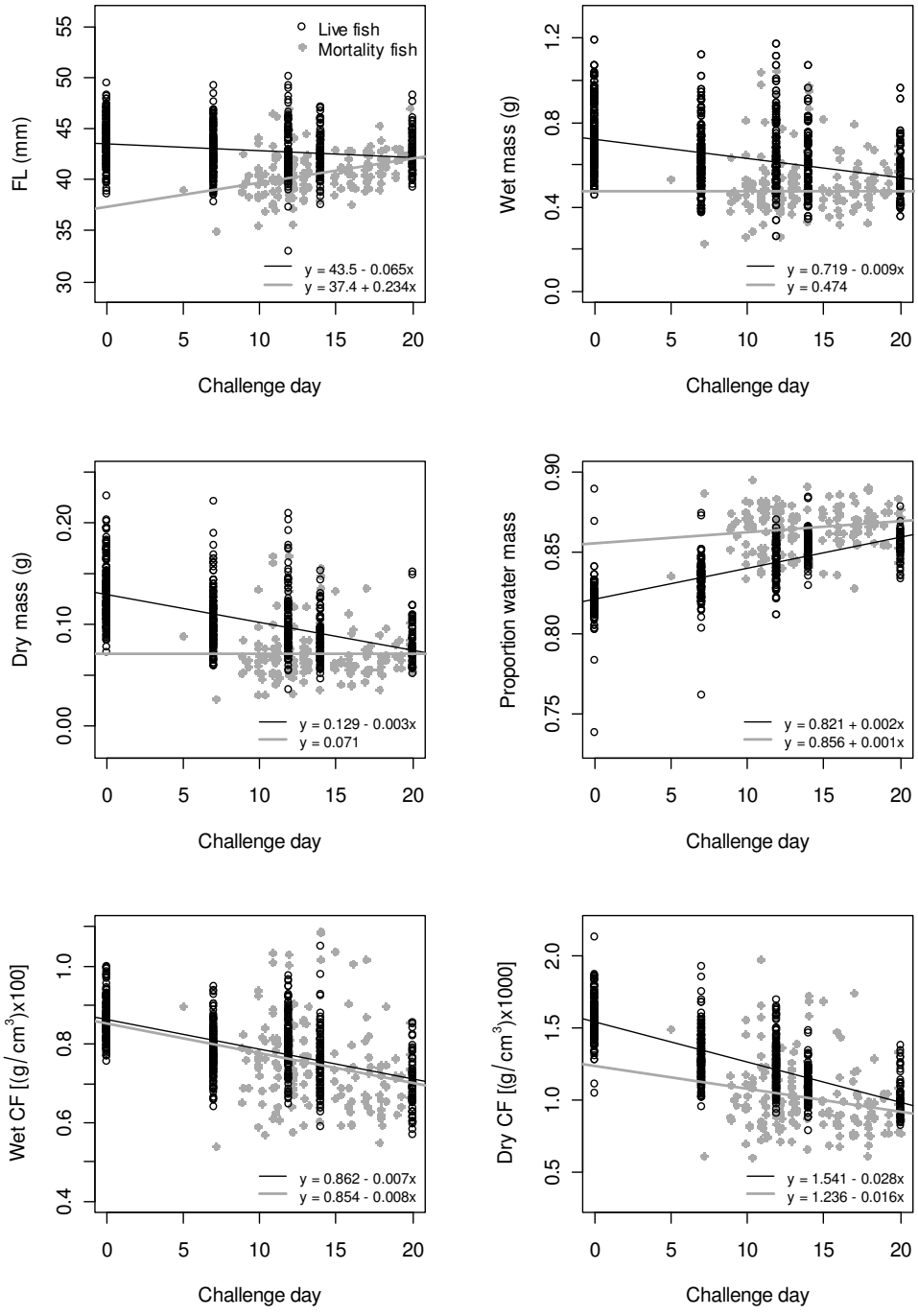

Figure 5.1. Relationship between fish condition indices and time for "Live" and "Mortality" fish. All linear relationships were statistically significant ($p < 0.05$). Black lines represent regression lines for "Live" fish, and gray lines represent regression lines for "Mortality" fish.

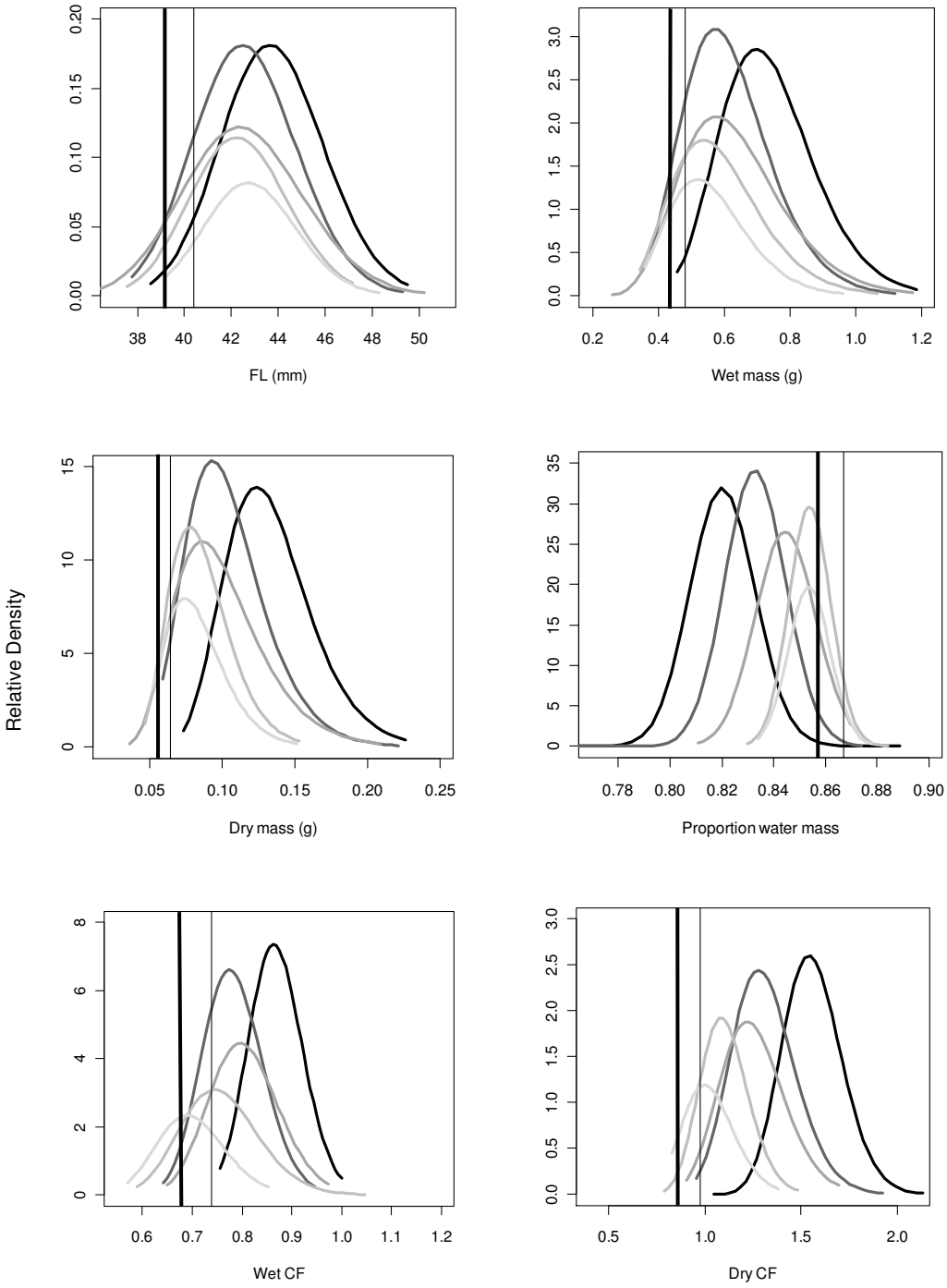

Figure 5.2. Inverse Gaussian distribution of fish condition indices of "Live" juvenile Coho salmon over the course of the challenge experiment. Subsamples 1 to 5 are represented by dark to light colored lines respectively. In each plot, thick vertical line represents 25th percentile and the thin vertical line represents the median of fish condition indices observed for "Mortality" fish.

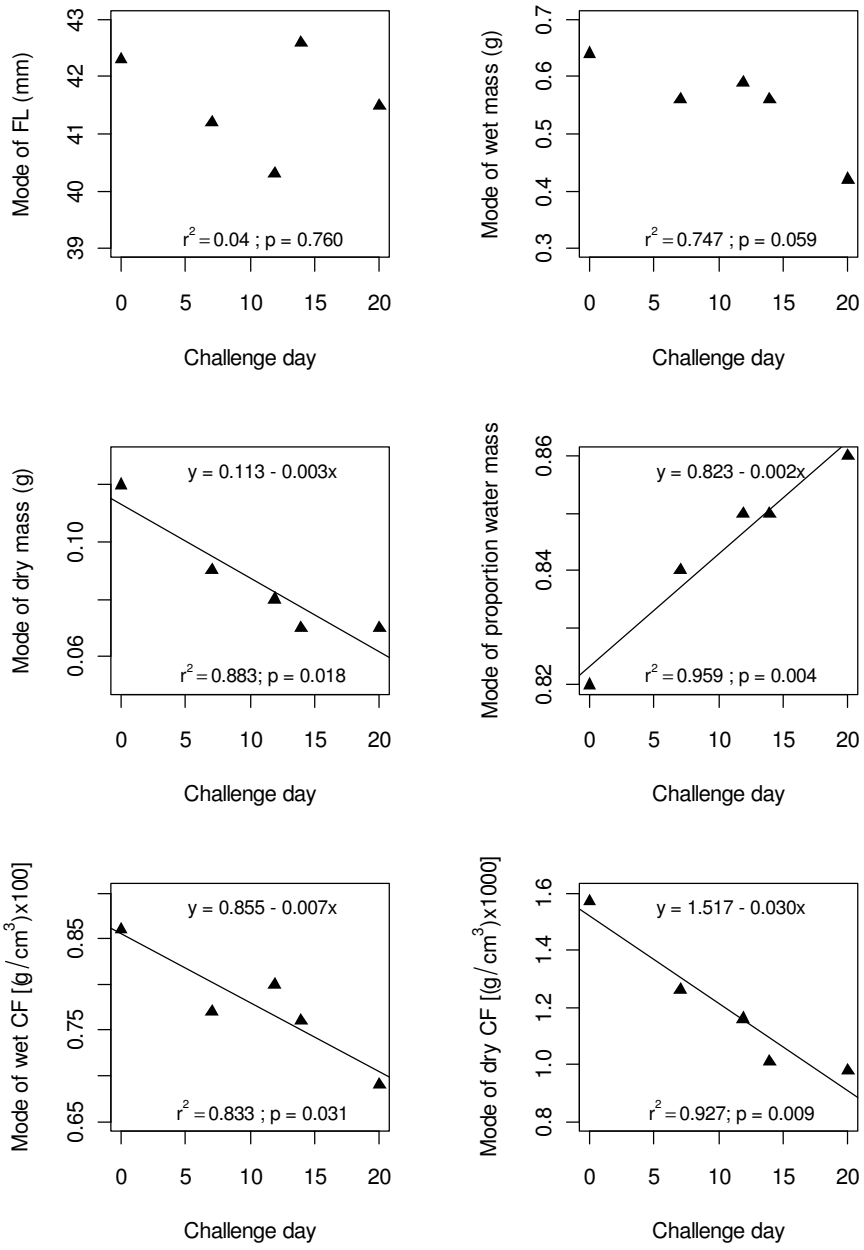

Figure 5.3. Mode of fork length, wet mass, dry mass, proportion water mass, wet CF, and dry CF of "Live" juvenile Coho salmon by challenge day. Regression lines plotted only for those with statistical significance of one-tailed test.

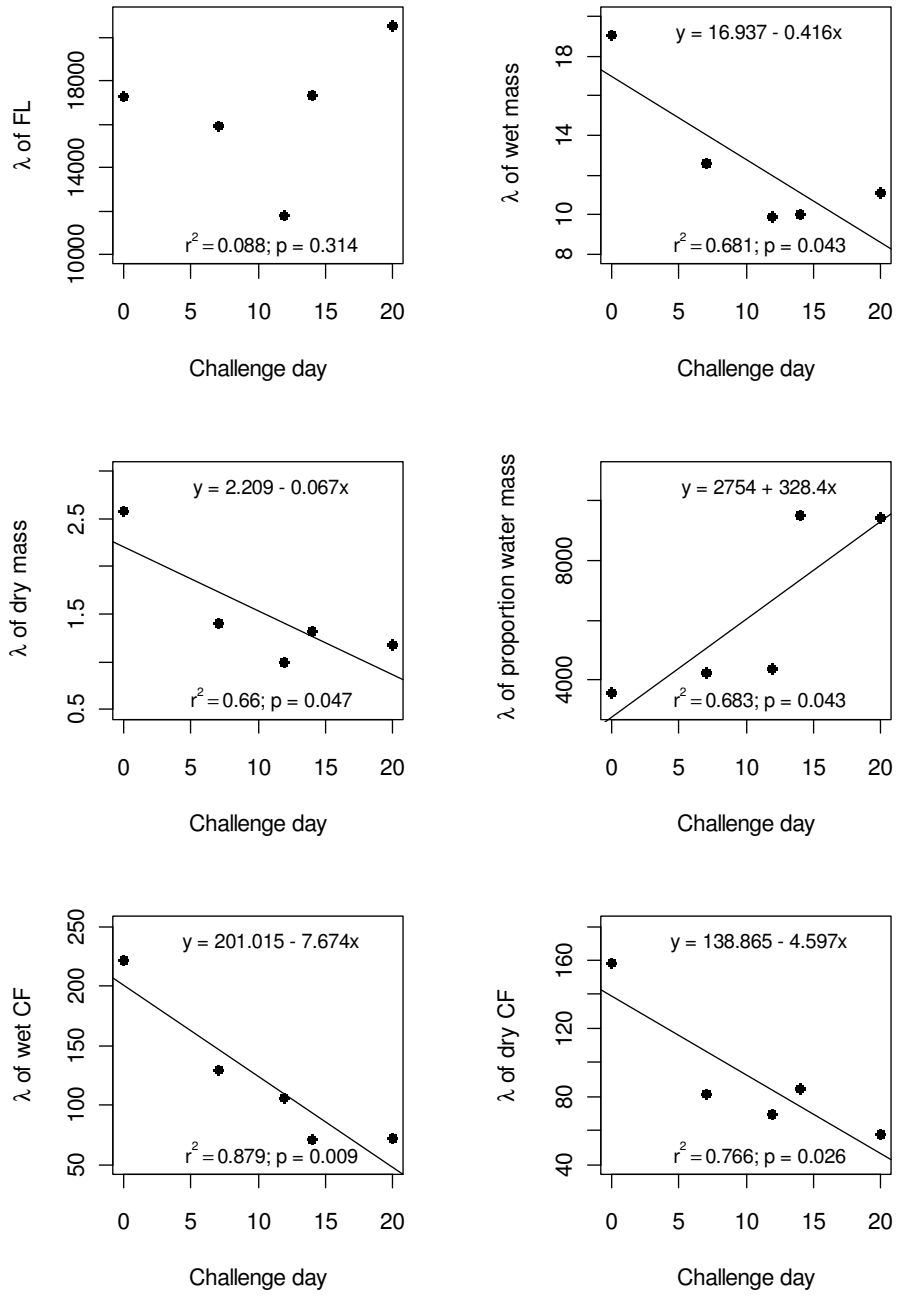

Figure 5.4. Shape parameter (λ) of the Inverse Gaussian distribution fitted to the fork length, wet mass, dry mass, proportion water mass, wet CF, and dry CF of "Live" juvenile Coho salmon by challenge day. Regression lines plotted only for those with statistical significance of one-tailed test.

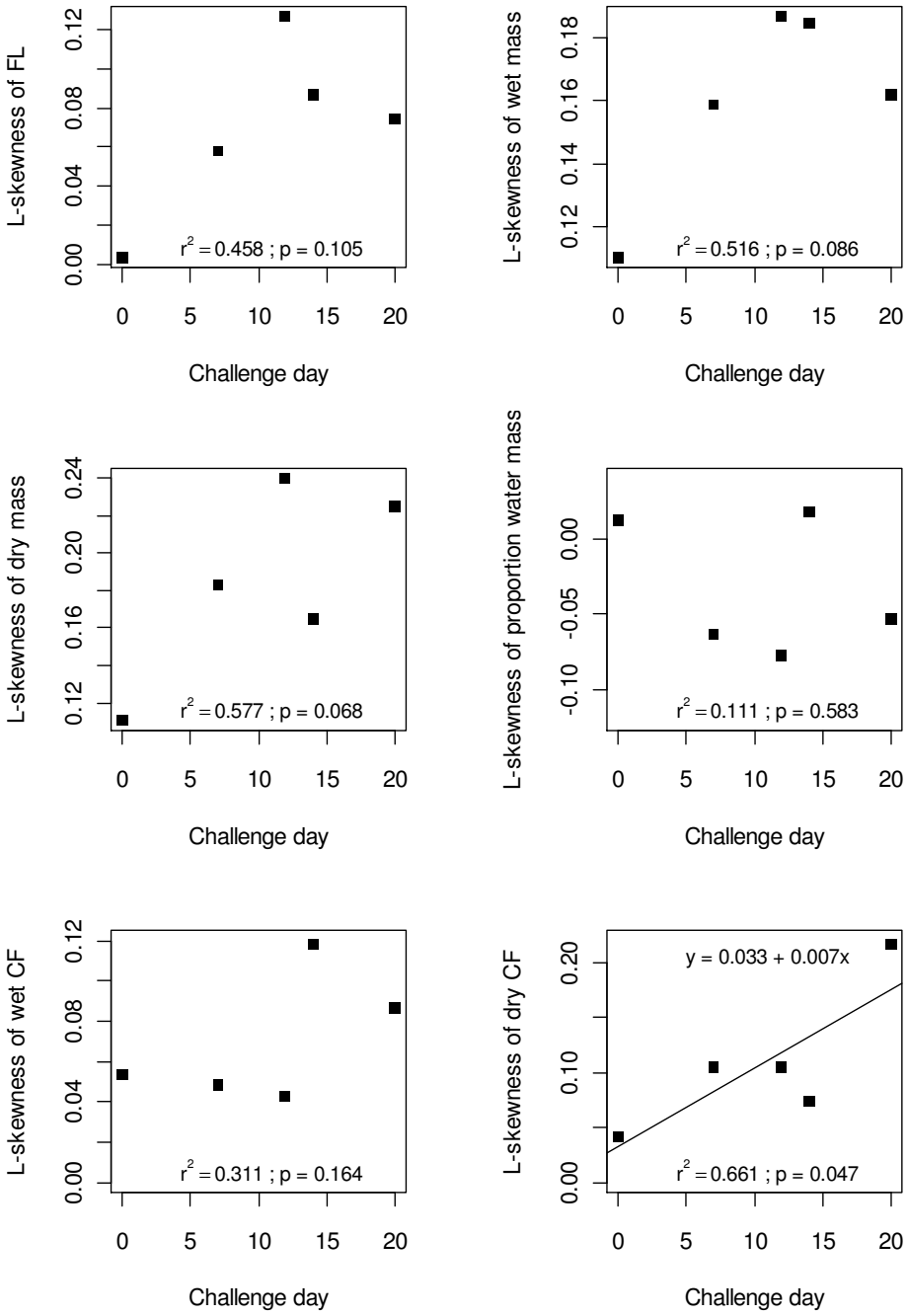

Figure 5.5. L-skewness of fork length, wet mass, dry mass, percent water mass, wet CF, and dry CF of juvenile Coho salmon still alive on day of sampling during the challenge. Regression line plotted only for the relationship with statistical significance of one-tailed test.

Discussion

Wet condition factor was the only fish condition index among the ones tested (FL, wet mass, dry mass, proportion water mass, wet CF, and dry CF) that did not differentiate between "Live" and "Mortality" fish in the challenge experiment. Other studies have also found lack of support for wet CF as an accurate predictor of fish condition (Trudel et al. 2005, Peters et al. 2007). The misrepresentation of wet CF as an accurate fish condition index may be two-fold: firstly, water contributes to body mass but is essentially void of energy density and secondly, excessive water mass is a sign of stress. Disruption of osmoregulatory ability is a possible mechanism that caused wet CF to not accurately represent the fish's condition in my study. Wet CF may not always be an appropriate fish condition index, but can be in some cases (Kaufman et al. 2007).

Among the fish condition indices standardized to mass (i.e. proportion water mass) or standardized to fork length (i.e. wet CF and dry CF), the critical thresholds progressed in a manner that supported a pattern of "Mortality" fish exhibiting greater tolerances to the sustained poor conditions. In contrast, a constant critical threshold has been observed in other studies (Letcher et al. 1996a, Crossin et al. 2004). A critical threshold of energy density occurred at 4 $MJ \cdot kg^{-1}$ in adult sockeye salmon during their upstream migration and post-spawning/moribund states (Crossin et al. 2004, Rand et al. 2006). Letcher et al. (1996a) found that the starvation threshold, which is the mass at lethal starvation divided by the maximum mass previously experienced, of yellow perch was constant. Furthermore, the constant critical thresholds of wet and dry mass (i.e. non-standardized fish condition indices) observed in my study also suggest that the later surviving fish were more tolerant of the challenge conditions. Because metabolic rates are greater in small-sized organisms than large-sized organisms (Sogard 1997, Gillooly et al. 2001), the small fish in my study were likely dying sooner than large fish

because they depleted their energetic reserves sooner. Additionally, with percent dry mass used as a proxy for energy density (Hartman and Brandt 1995), dry mass of dead fish is expected to increase throughout the challenge if there was a constant critical threshold of energy density. The constant dry mass and wet mass observed in the "Mortality" fish in my study suggest that the surviving fish were increasingly more tolerant of the challenge conditions and were depleting their reserves more as the challenge progressed relative to the fish that died earlier in the challenge. Together, the patterns of constant wet and dry mass, the decreasing wet and dry CF, and the increasing proportion water mass of the "Mortality" fish suggest that they were increasingly more tolerant of the challenge conditions. The sloped critical thresholds observed for the standardized fish condition indices suggest that differences among individuals in their critical thresholds may have occurred. Heterogeneity among individuals is common, and differences among individuals in the critical threshold may also be possible. Varying physiological tolerances and varying susceptibility to disease have been observed (Wedemeyer et al. 1980, Johnson and Evans 1996, West and Larkin 1996). An alternative, but not exclusive, hypothesis is that smaller sized fish were affected by other stressors not recorded in my study. I recorded physical, but not physiological or pathological, characteristics of the fish. Although the fish may still have a constant critical threshold for each standardized fish condition index, the time to reach a multivariate critical threshold is lower in small fish than large fish. Overall, a constant threshold for a single condition index may not always be appropriate, and a multivariate condition index that incorporates allostasis, homeostasis, and disease warrants further investigation. This is particularly important because I was assuming a direct correlation between mortality and my fish condition indices.

Among the three statistical indices examined, each one encapsulated different aspects of the fish condition indices. The mode captured the deteriorating conditions of the fish, while L-

skewness depicted the shape of the distribution. The λ parameter of an IG distribution, which incorporates the mean and standard deviation, represented both the deteriorating conditions of the fish and the shape of the distribution. However, for λ to fully describe the shape of the distribution, it is only meaningful in context of the mean of the fish condition index measurements. The mode and λ are thus useful in observing declining fish conditions in longitudinal but not snapshot datasets. Skewness is convenient in that it can be compared to zero. The values of skewness observed in my samples were relatively low and thus would have required larger sample sizes for sufficient statistical power. The observed L-skewness values of approximately 0.2 are comparable to other skewness indices observed in other studies (Weis et al. 1992, De Soyza et al. 1997) and described in a textbook example (Underwood 1997). Despite the relatively low skewness observed in my study, an increasing pattern of L-skewness was significantly detected in dry CF, and trended in fork length, wet mass, and dry mass. Higher degrees of skewness may only occur at higher rates of mortality, or in a group with greater initial heterogeneity. Despite, theoretical descriptions of skewed distributions in ecology (Underwood 1997, Rand et al. 2006) and empirically observed critical thresholds of populations under lethal stress (Crossin et al. 2004, Rand et al. 2006), few studies have reported observed skewed distributions in organisms of deteriorating conditions (Orfanidis et al. 2010). Skewness has been tested, along with the Shapiro-Wilk statistical test for normality, to examine major changes in deteriorating fish conditions such as Atlantic cod (*Gadus morhua*) (Dutil et al. 2003). But this study did not observe Atlantic cod to be under lethal stress in their year of study. Overall, in my study, there is weak support, but not rejection, of L-skewness as an indicator for a population under lethal stress based on its significant patterns and non-significant trends throughout my challenge experiments. Future studies could re-examine skewness with varying degrees of heterogeneity among the population and higher rates of mortality.

Other future studies could investigate the relationship of a multivariate fish condition index to L-skewness. As discussed earlier, any one fish condition index may not be sufficient in characterizing a fish's condition because multiple physical, physiological, and pathological processes may be occurring. In my study, I used "challenge day" as a proxy for overall fish condition. Mortality rate is not an appropriate fish condition index because fish can deteriorate in condition but not yet die. Vitality can be an appropriate representation of a multivariate fish condition index (Anderson 2000, Anderson et al. 2008, Li and Anderson 2009), however it requires complete, or nearly complete, survival curves for parameter estimation which is not always feasible. A multivariate fish condition index (or composite variable) has also been suggested by Wagner and Congleton (2004).

Different ecological processes can cause skewed patterns of indices at both the level of the individual and the community (Weis et al. 1992, Underwood 1997, Orfanidis et al. 2010). At the level of individuals in a population, examples of underlying mechanisms include starvation and predation. With starvation, there is a critical lower threshold of a energetic reserves beyond which few individuals survive (Letcher et al. 1996a, Biro et al. 2004, Crossin et al. 2004, Rand et al. 2006). With size-selective predation there is a critical lower threshold that prey must reach before they are susceptible to predation (Connell 1970), or more often a critical upper threshold in which prey become safe from predation (Sogard 1997). Starvation is a bottom-up process and predation is a top-down one. Furthermore, these bottom-up and top-down processes could affect multiple species in addition to the target species. Indicators based on skewness have been developed to assess the health of communities and ecosystems (De Soyza et al. 1997, Reizopoulou and Nicolaidou 2007). Conceptually, this is comparable to scaling up from "individuals of a population" to "species of a community". In one study, a sensitive indicator of disturbance to a desert ecosystem was determined as the skewness of the frequency distribution of

ln-transformed bare soil patch size weighted by mean bare patch size (De Soyza et al. 1997). In another study, the observed right-skewed geometric size (or biomass) classes indicated that a coastal lagoon ecosystem had a low Ecological Quality Status (EQS; Reizopoulou and Nicolaidou 2007). Small-sized individuals across multiple species were favored in the ecosystem with low EQS because of high levels of organic carbon in the sediment and low dissolved oxygen. Skewed distributions of an organism's condition index can result from different ecological processes, and may be indicative of processes occurring at a level beyond that of the population of one species. It is thus important to understand the underlying processes and their possible breadth of effect across the community and ecosystem.

In summary, the deteriorating condition of the fish during the challenge was detected in the six indices tested. My results also suggest that dry CF is a more accurate fish condition index than wet CF under lethally stressful conditions. The patterns in the critical threshold of the six fish condition indices suggest that the remaining live fish in the challenge were increasingly tolerant of the challenge conditions. Thus, a multivariate fish condition index warrants further investigation. L-skewness of dry CF is a possible, but potentially insensitive, indicator of a population under lethal stress. For this reason, even though L-skewness can be a fish condition index that does not require longitudinal datasets, pairing it with historical data or an estimate of the critical threshold would help improve interpretations of the population's condition than either type of measure alone.

Chapter 6

Conclusions, Implications and Future Directions

The effects of past experiences on current survival capacities can be particularly important in organisms with life history stages across different environments. As demonstrated in my four main research studies, the past conditions experienced (e.g. level of food availability and presence or absence of competition; type of passage through a hydropower system and associated environmental conditions) resulted in differences among individuals in their fish conditions and hence their survival capacities as they confront current and future challenge conditions.

In Chapter 2, I found that heterogeneity among individuals helps buffer against mortality. The general concept underlying this process is similar to the portfolio effect of high species biodiversity and high diversity in life history types (Hooper et al. 2005, Schindler et al. 2010). The difference is that I studied it at the level of individuals within one group (or population), in one physical trait (mass) as well as one abstract trait (vitality) that encompasses all traits of an individual. My work supports the findings found in other research studies in which variability among individuals in growth increases survival at the population level (Rice et al. 1993, Letcher et al. 1996b); but it is the first to determine that competition-induced heterogeneity has beneficial effects to population survival at a later time in life. My results and revelation of some underlying processes further emphasize the need to maintain heterogeneity within the population for species conservation (Hooper et al. 2005, Schindler et al. 2010).

Again, in Chapter 3, the importance of past experiences and heterogeneity among individuals appears in my study of step-like patterns in a survival curve resulting from thermal stress over the short term and starvation over the long term. This further supports the need for life

cycle models and the incorporation of past conditions experienced, heterogeneity among individuals, and current natural selection processes.

In the Federal Columbia River Power System (FCRPS; Washington and Oregon, USA), differences between the threatened (NMFS 2011) run-of-river (ROR) juvenile hatchery spring/summer Chinook salmon and their barged counterparts from a mitigation strategy (Juvenile Fish Transportation Program, U.S. Army Corps of Engineers) have been found in their post-FCRPS smolt-to-adult return rates (Anderson et al. 2005, NOAA 2010, Tuomikoski et al. 2010). I was also able to detect differences in survival capacities between these ROR (lower survival capacity) and barged fish (higher survival capacity), but primarily in their seasonal patterns (Chapter 4). Other research studies have found strong seasonal patterns with hypothesized selection processes in the estuary and ocean (Scheuerell and Williams 2005, Muir et al. 2006, Schreck et al. 2006, Eder et al. 2009a, Scheuerell et al. 2009, McMichael et al. 2010b), but this study is the first to detect a seasonal pattern of survival capacity before migration through post-FCRPS environments. The influences of water temperature on biological and ecological processes such as survival, behavior, geographical distribution, metabolism and growth are pervasive in the literature. It is thus not surprising that the water temperature previously experienced by ROR and barged fish affected their post-FCRPS survival capacities. At a time of climate change and alteration of management strategies in the spill-transport program (ISAB 2008b), it is important to understand the underlying processes of survival linked to water temperature and other seasonal conditions experienced by the fish as they pass through the FCRPS and the selection processes they face after the hydropower system.

From my work in Chapter 5, I can see first-hand how the quest in determining a time- and cost-effective, but yet informative, measure of fish condition with snapshot data continues. Although my study with juvenile hatchery Coho salmon provided some evidence of L-skewness

being an indicator of lethal stress and mortality occurring at the population level, it may only be a useful indicator at high rates of mortality. Because this hypothesis of increased skewness due to mortality of the weakest individuals is assumed in research studies (Rand et al. 2006, Li and Anderson 2009) and demonstrated in simulation modeling (Finstad et al. 2004), it warrants further investigation. Furthermore, the pattern of an increasingly lower critical threshold in single fish condition indices as mortality rate increases suggests that a more encompassing index such as a multivariate fish condition index or vitality may be more appropriate.

Individuals continually experience environmental and ecological conditions that cumulatively affect their intrinsic conditions and hence current and future survival capacities. A recently published paper (Healey 2011) painted a rather grim outlook for anadromous Pacific salmon due to cumulative effects of climate change across the life stages of egg and alevin, fry entering nursery lakes, fry during their first winter, smolts during seaward migration, postmolts in the estuary and coastal ocean, immatures in the ocean, and returning adults. Temperature effects on growth, development, and the timing of emergence and migration can result in increased size-predation risk and mismatch with timing of prey resources that are compounded from one life stage to the next. Healey (2011) notes that his model is only a qualitative model, and that quantitative models would help clarify how changes in responses at one life stage propagate through subsequent life cycles and even subsequent generations. Because survival has many underlying processes, its complexity may be more manageable, but still analytically accurate, by using an abstract measure such as vitality (Anderson 2000, Anderson et al. 2008, Li and Anderson 2009). Coupling vitality modeling with some biological indices will provide a comprehensive and mechanistic-based understanding of survival. One such index could be energetic reserves because of its close relationship to survival capacity through allostasis, a process in which all biological processes within an individual decrease or increase its energetic

reserves (McEwen and Wingfield 2003). My dissertation is timely in providing insights to underlying processes of cumulative effects on cross-life stage survival because of the current research needs and impacts of climate change (Healey 2011). This broad concept of cumulative effects is also relevant in the management of natural resources such as ESA-listed stocks of salmon as demonstrated in my research. As a whole, this dissertation provides further motivation for research on survival and management of living resources to incorporate cross-life stage effects and heterogeneity among individuals.

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APPENDIX A

Supplemental Information on Methods in the Challenge Experiments Conducted on Guppies for Chapter 2

Aquarium water quality: The water in the recirculating aquarium system with parental guppies was conditioned with Start Right, Freshwater Essentials, and Stresscoat, and live nitrifying bacteria were established with Dr. Tim's Aquatics One & Only, Ammonium Chloride, Proline Water Conditioner, and Proline Bio-Booster Trace Elements & Vitamins. The water quality was monitored and maintained daily with the following indices (concentrations in parentheses): ammonia (0 ppm), nitrite (0 ppm), nitrate (0 ppm) dissolved oxygen (8 to 10 ppm), GH (5 to 6 ppm), and KH (5 to 6 ppm). The water temperature was measured by temperature loggers (iButton® 1-wire Thermocron in waterproof capsules) at 10-minute intervals, and averaged 22.3°C ± SD 0.5°C (N=14,255). Lighting was provided by fluorescent lights from the ceiling on a 12:12 light:dark cycle. Tanks were arranged with gravel and plastic aquarium plants to allow refuge for the guppies. For guppies in the treatment stage, the static tanks were equipped only with aerators and in some tanks temperature loggers. The water quality monitoring and maintenance was the same as those for the tank system with parental guppies except for the following indicators (average concentrations in parentheses): ammonia (0.1 ppm), nitrite (0.03 ppm), and dissolved oxygen (8 ppm). For guppies in the challenge stage, the water quality monitoring and maintenance was the same as those for the tank system with parental guppies except for the dissolved oxygen which averaged 8 ppm.

Conversion of brine shrimp quantity: When feeding the guppies brine shrimp, the amount was first quantified as the number of individuals and later converted to mass. This was accomplished

by pipetting 20 µL from a 520 mL mixture of tank water and a measured amount of brine shrimp in grams, counting the number of individuals in the 20 µL, and extrapolating to determine the mass of one brine shrimp. The mass of brine shrimp fed to guppies was then determined by multiplying the estimated individual brine shrimp mass with the number of individuals fed on a daily basis.

Challenge water temperatures: The 8-L challenge tanks contained a temperature logger and a submersible heater. Water temperatures were recorded every 10 minutes with temperature loggers. I intentionally increased the water temperature of the tanks progressively gradually over four days to allow acclimation and to minimize stress experienced when nearing the target temperature. The water temperature was increased from 21.5°C to 28°C on the first day, to 31°C on the second day, to 32°C on the third day, and to 33°C on the fourth day at which time the challenge stage began. The temperature during the challenge stage was 33.3°C ± 0.96°C (N=11,114) across all replicate tanks.

APPENDIX B

Supplemental Information on Methods of Challenge Experiments Conducted on Rainbow Trout for Chapter 3

Appendix B.1. Water quality, lighting, and temperature conditions

Water quality was measured daily throughout the challenge and averaged as follows: pH = 7.0 ± SE 0.3, [NO_3] = 0.6 ppt ± SE 0.1 ppt, [NH_3/NH_4] = 0.2 ppt ± SE 0.05 ppt, and DO = 94.5% ± SE 0.6%. The light:dark cycle in the laboratory was set to follow the natural light at 8h:16h. The water temperature was gradually increased by turning off the chiller and warmed by room temperature over three days at a rate of approximately 0.2°C/hour until the target temperature of 24°C was reached (Appendix Figure 3.1). The water temperature was recorded every 10 minutes by temperature loggers (Maxim iButton® DS1922) in waterproof capsules and once a day by a temperature and dissolved oxygen meter. The water temperature from the onset of the challenge, when 24°C was reached, to the end of the challenge, when no fish remained, averaged 23.9°C + SE 0.05°C.

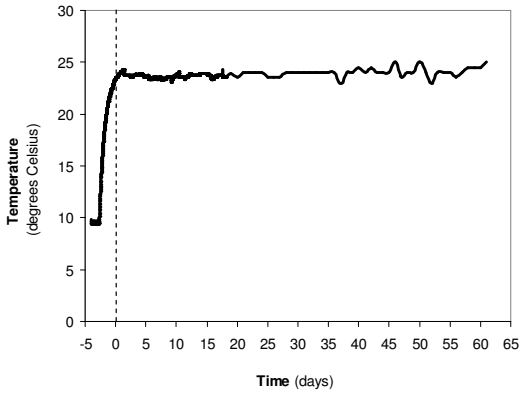

Figure A.1. Water temperature recorded five days prior to and throughout the challenge experiment.

Appendix B.2. Fish densities in the aquarium system

At the beginning of the experiment, fish densities were 0.2 individuals·L^{-1}. As rainbow trout died throughout the challenge, the densities decreased. To minimize the effects of varying densities and the effects of handling on the rainbow trout, fish were regrouped into fewer tanks once throughout the challenge. This occurred on day 45 of the challenge, when fish in the Mortality group from tank #2 were moved to tank #1, and fish in tank #4 were moved to tank #3. Fish densities in the four tanks were respectively 0.1, 0.05 , 0.03, and 0.05 individuals·L^{-1}. The new densities in the two tanks were 0.1 individuals·L^{-1}. For the LOE group, the densities for the four tanks were 0.08, 0.1, 0.1, and 0.03 individuals·L^{-1} respectively. These fish were regrouped into one tank and the new density was 0.1 individuals·L^{-1}.

Appendix B.3.Two-process vitality model (Li et al. *In preparation*)

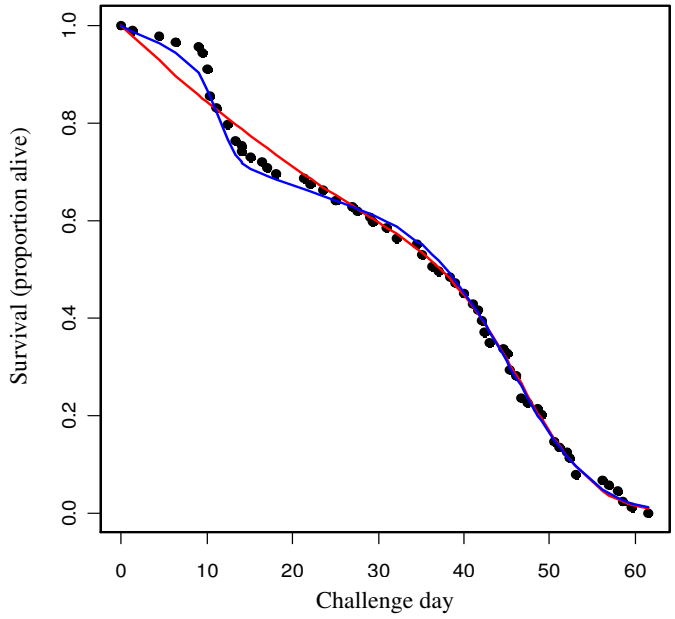

Figure A.2. Time to mortality of juvenile rainbow trout exposed to two stressors of increased water temperature and absence of food resources. Vitality model fits are by the one-process selection version (blue line; Li and Anderson 2009) and the two-process selection version (red line; Li et al. *In preparation*).

APPENDIX C

Supplemental Information on Methods of Challenge Experiments Conducted on Chinook Salmon for Subchapter 4A

Appendix C.1. Water quality of challenge experiments

Water quality tests were conducted daily for each tank. Water exchanges, in addition to the flow-through system (approximately 50 $L \cdot h^{-1}$), were conducted to maintain ammonia and nitrite levels low. The water quality measures throughout the study across all tanks and challenge tests were as follows: pH = 7.2 ± SE 0.005, [NO_3] = 0.1 ppt ± SE 0.02 ppt, [NH_3/NH_4] = 0.8 ppt ± SE 0.5 ppt, and DO = 8.0 ppt ± SE 0.1 ppt. Water temperatures during the challenges were measured at 10 minute increments and averaged 24.3°C + 0.01°C.

Appendix C.2. Elimination of highly correlated predictors from the full multi-linear model

Among ten potentially correlated predictors of survival capacity measures, water temperature at barge loading site or collection site after ROR migration (T_{site}) and barge index (B; 1=barged fish, 0=ROR) were chosen to be included in the full multi-linear models. The other predictors considered were water temperature at Bonneville Dam (T_{BON}), travel time from LGR to BON ($\hat{t}_{LGR}$), travel time from three select points of entry of the FCRPS to BON ($\hat{t}_{FCRPS}$), day-of-year of sampling at BON (DOY_{BON}), DOY at time of entry into the FCRPS ($\hat{DOY}_{FCRPS}$), DOY at time of passage through LGR ($\hat{DOY}_{LGR}$), and two types of estimates of degree days ($\hat{Q}$ and $\hat{q}$).

The $\hat{t}_{FCRPS}$ values were estimated by first querying all juvenile hatchery spring/summer Chinook salmon in the PTAGIS database (ptagis.org) for travel times from MCN to BON with

the arrival date as the day of sampling +/- 1 day. The average travel time from MCN to BON was subtracted from DOY_{BON} to determine the average day of arrival at MCN. The travel time from LMN to MCN and from LGR to LMN were determined in a similar manner. Travel times starting from LGR, MCN, and nearby but above BON were weighted by $\hat{P}_{LGR}$, $\hat{P}_{MCN}$, and $\hat{P}_{BON}$ respectively. The $\hat{t}_{LGR}$ values were thus determined in the process of estimating $\hat{t}_{FCRPS}$.

$$\hat{t}_{FCRPSi} = \hat{P}_{LGRi}\hat{t}_{LGRi} + \hat{P}_{MCNi}\hat{t}_{MCNi} + \hat{P}_{BONi}\hat{t}_{BONi}$$

Where

$$\hat{t}_{LGR} = \overline{\hat{t}}_{MCN-BON} + \overline{\hat{t}}_{LMN-MCN} + \overline{\hat{t}}_{LGR-LMN}$$

$$\hat{t}_{MNC} = \overline{\hat{t}}_{MCN-BON}$$

$\hat{t}_{BON}$ = mean travel time from select hatcheries near Bonneville Dam to Bonneville Dam for salmon that passed BON on $DOY_{BON} \pm 1$ day

$\overline{\hat{t}}_{MCN-BON}$ = mean travel time from MCN to BON of all hatchery spring/summer Chinook yearlings in PTAGIS that passed BON on $DOY_{BON} \pm 1$ day

$\overline{\hat{t}}_{LMN-MCN}$ = mean travel time from LMN to MCN of all hatchery spring/summer Chinook yearlings in PTAGIS that passed MCN on $\hat{DOY}_{MCN} \pm 1$ day

$\overline{\hat{t}}_{LGR-LMN}$ = mean travel time from LGR to MCN of all hatchery spring/summer Chinook yearlings in PTAGIS salmon that passed LMN on $\hat{DOY}_{LMN} \pm 1$ day

$$\hat{DOY}_{LGR_i} = \hat{DOY}_{LMN_i} - \overline{\hat{t}}_{LGR-LMN_i}$$

$$\hat{DOY}_{LMN_i} = \hat{DOY}_{MCN_i} - \overline{\hat{t}}_{LMN-MCN_i}$$

$$\hat{DOY}_{MCN_i} = DOY_{BON_i} - \overline{\hat{t}}_{MCN-BON_i}$$

$$\hat{t}_{LGR-BON} = \hat{DOY}_{LGR_i} - \hat{DOY}_{MCN_i}$$

P = Proportion of sample by first FCRPS dam encountered (LGR, MCN, or BON)
t = Travel time (days)
i = sample
See Methods of Chapter 4A for $\hat{P}_{LGR}$, $\hat{P}_{MCN}$, and $\hat{P}_{BON}$.

$\hat{DOY}_{FCRPS}$ and $\hat{DOY}_{LGR}$ were estimated by subtracting travel times $\hat{t}_{FCRPS}$ and $\hat{t}_{LGR}$ respectively from DOY_{BON}. The degree days $\hat{q}$ was estimated as the cumulative water temperature

experienced since their FCRPS entry either at LGR, MCN or BON, weighted by $\hat{P}_{LGR}$, $\hat{P}_{MCN}$, and $\hat{P}_{BON}$.

$$\hat{q}_i = \hat{P}_{LGRi}\hat{q}_{LGRi} + \hat{P}_{MCNi}\hat{q}_{MCNi} + \hat{P}_{BONi}\hat{q}_{BONi}$$

Where

$$\hat{q}_{LGR} = \hat{T}_{LGR-LMN}\hat{t}_{LGR-LMN} + \hat{T}_{LMN-MCN}\hat{t}_{LMN-MCN} + \hat{T}_{MCN-BON}\hat{t}_{MCN-BON}$$

$$\hat{q}_{MCN} = \hat{T}_{MCN-BON}\hat{t}_{MCN-BON}$$

$$\hat{q}_{BON} = \hat{T}_{BON}\hat{t}_{BON}$$

$$\hat{T}_{LGR-LMN} = \frac{T_{LGR} + T_{LMN}}{2}$$

$$\hat{T}_{LMN-MCN} = \frac{T_{LMN} + T_{MCN}}{2}$$

$$\hat{T}_{MCN-BON} = \frac{T_{MCN} + T_{BON}}{2}$$

q = degree days (°C × days)
P = Proportion of sample by first FCRPS dam encountered (LGR, MCN, or BON)
T = river temperature (°C)
t = travel time (days)
i = sample

See Methods of Chapter 4 for $\hat{P}_{LGR}$, $\hat{P}_{MCN}$, and $\hat{P}_{BON}$.

$\hat{Q}$ was estimated as $\hat{q}$ plus the cumulative water temperature experienced since March 25 at either LGR, MCN, or BON, whichever was considered to be their first point of entry into the FCRPS. Daily average water temperatures at LGR, MCN, or BON were summed from March 25 to the time of their respective dam passage. $\hat{Q}$ is the sum of this type of degree days across the three select dams weighted by $\hat{P}_{LGR}$, $\hat{P}_{MCN}$, and $\hat{P}_{BON}$ respectively.

$$\hat{Q}_i = \hat{P}_{LGR_i}\hat{Q}_{LGR_i} + \hat{P}_{MCNi}\hat{Q}_{MCN_i} + \hat{P}_{BON_i}\hat{Q}_{BON_i}$$

Where

$$\hat{Q}_{LGR} = \sum_{j=Mar\,25}^{\hat{J}_{LGR}} T_{LGR_j} + \hat{q}_{LGR}$$

$$\hat{Q}_{MCN} = \sum_{j=Mar\,25}^{\hat{J}_{MCN}} T_{MCN_j} + \hat{q}_{MCN}$$

$$\hat{Q}_{BON} = \sum_{j=Mar\,25}^{J_{BON}} T_{BON_j} + \hat{q}_{BON}$$

i = sample

Most of the predictors were highly correlated to each other (Table IIIA.1). T_{site} had the highest percent of variation in survival capacity ln(m) and ln(r) explained in the hierarchical partitioning analysis which considers each explanatory variable tested as orthogonal (i.e. independent) to each other (Figures IIIA.1 and IIIA.2). The barge index was also chosen to include into the full multi-linear models of survival capacity because it is essentially the two treatment groups tested, and it is not highly correlated to T_{site} (Table IIIA.1). The travel time indices were also not highly correlated with T_{site} but were highly correlate with B. The barge index was chosen over the travel time indices because it was a more accurate measure. The degree days $\hat{q}$ was also not highly correlated with T_{site}, but because these two variables are conceptually a similar type of measure $\hat{q}$ was not tested in the full model.

Table A.1. Correlation coefficients between potentially highly correlated factors. N=35 except correlation tests with $\hat{DOY}_{LGR}$ and $\hat{t}_{LGR}$ which had N=33.

	DOY_{BON}	$D\hat{O}Y_{FCRPS}$	$D\hat{O}Y_{LGR}$	T_{site}	T_{BON}	$\hat{Q}$	$\hat{q}$	B	$\hat{t}_{LGR}$	$\hat{t}_{FCRPS}$
DOY_{BON}	**1.000**	0.915	0.850	0.820	0.950	0.982	0.347	-0.065	0.175	0.161
$D\hat{O}Y_{FCRPS}$	0.915	**1.000**	0.981	0.610	0.875	0.914	-0.039	0.273	-0.231	-0.245
$D\hat{O}Y_{LGR}$	0.850	0.981	**1.000**	0.464	0.797	0.866	-0.163	0.357	-0.369	-0.330
T_{site}	0.820	0.610	0.464	**1.000**	0.844	0.775	0.663	-0.462	0.562	0.479
T_{BON}	0.950	0.875	0.797	0.844	**1.000**	0.939	0.324	-0.072	0.178	0.133
$\hat{Q}$	0.982	0.914	0.866	0.775	0.939	**1.000**	0.318	0.010	0.111	0.129
$\hat{q}$	0.347	-0.039	-0.163	0.663	0.324	0.318	**1.000**	-0.815	0.922	0.958
B	-0.065	0.273	0.357	-0.462	-0.072	0.010	-0.815	**1.000**	-0.945	-0.830
$\hat{t}_{LGR}$	0.175	-0.231	-0.369	0.562	0.178	0.111	0.922	-0.945	**1.000**	0.938
$\hat{t}_{FCRPS}$	0.161	-0.245	-0.330	0.479	0.133	0.129	0.958	-0.830	0.938	**1.000**

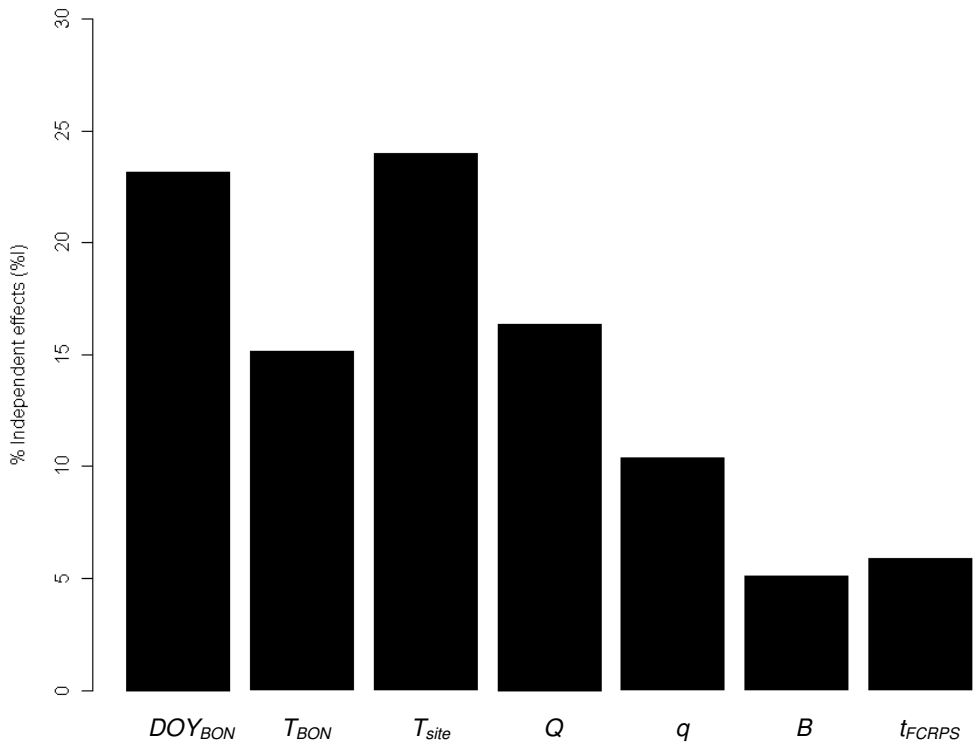

Figure A.3. Percent independent effects of potential factors of juvenile spring/summer Chinook survival capacity ln(m) analyzed by hierarchical partitioning. N = 35

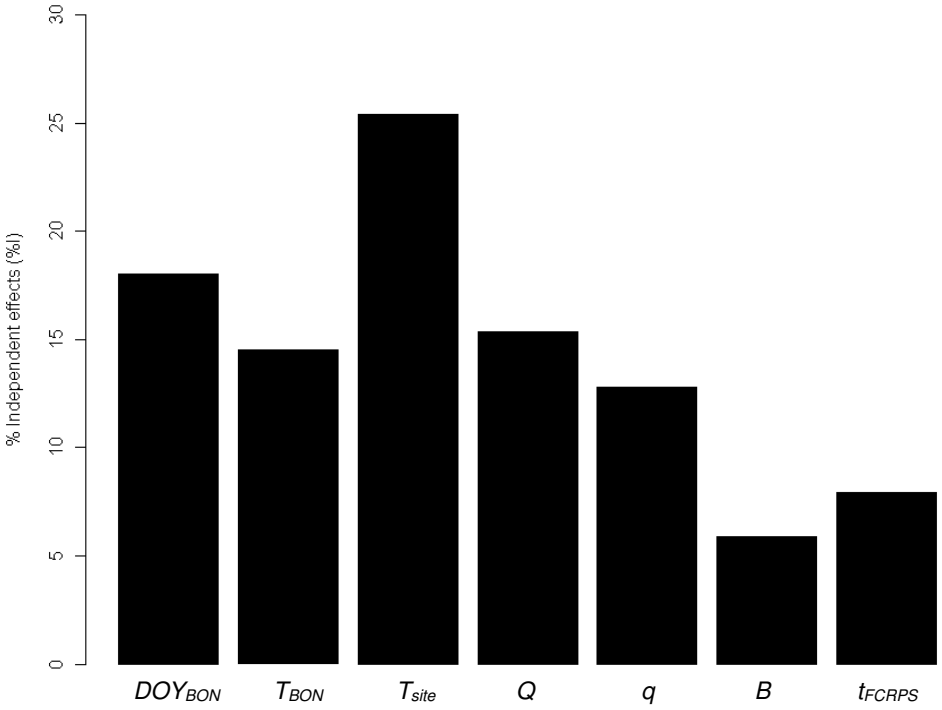

Figure A.4. Percent independent effects of potential factors of juvenile spring/summer Chinook survival capacity ln(r) analyzed by hierarchical partitioning. N = 30

Appendix C.3. Seasonal patterns of predictor variables

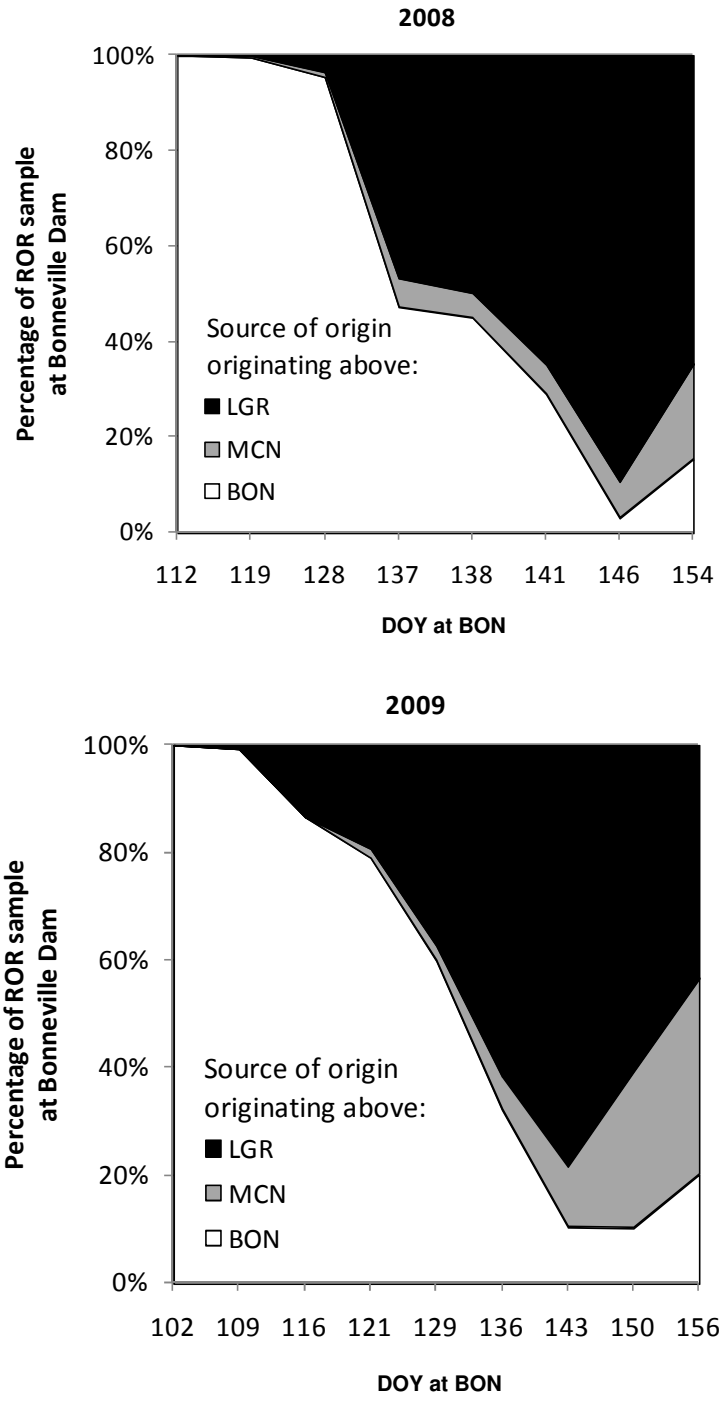

Figure A.5. Estimated proportion of ROR hatchery yearling Chinook salmon passing Bonneville Dam (BON) by first select FCRPS dam (LGR, MCN, or BON) encountered in 2008 (top) and 2009 (bottom).

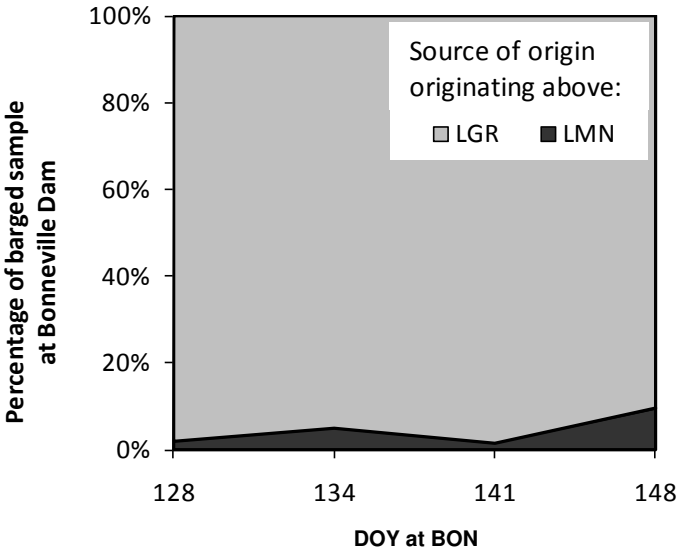

Figure A.6. Percentage of yearling Chinook salmon collected and barged from LMN to Bonneville Dam by first select FCRPS dam (LGR or LMN) encountered in 2009.

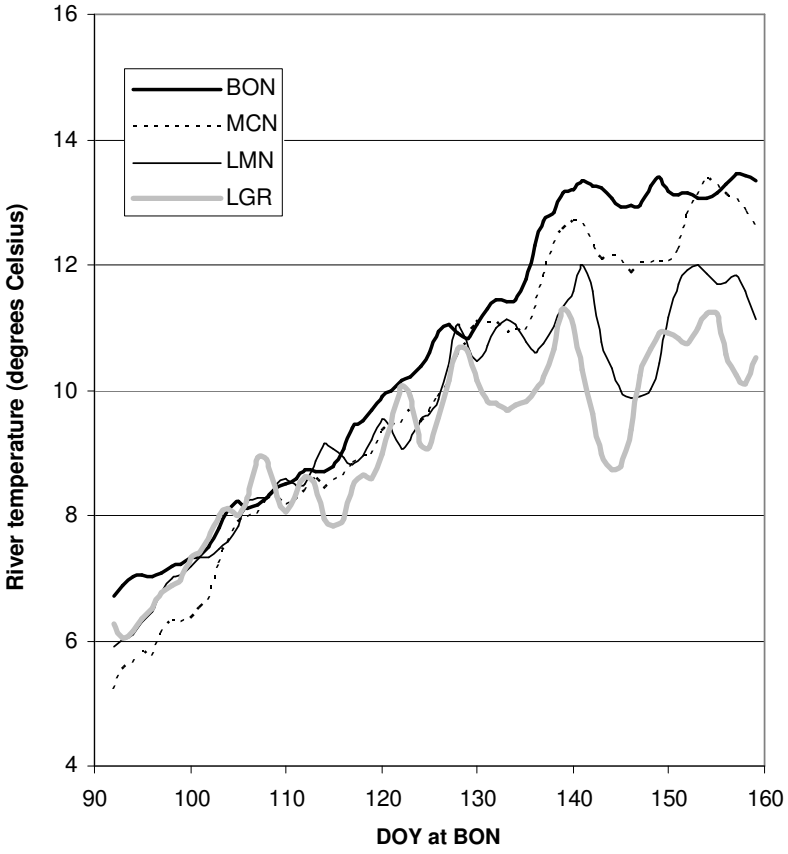

Figure A.7**.** River water temperature (°C) at Lower Granite Dam (LGR), Lower Monumental Dam (LMN), McNary Dam (MCN), and Bonneville Dam (BON) in 2008.

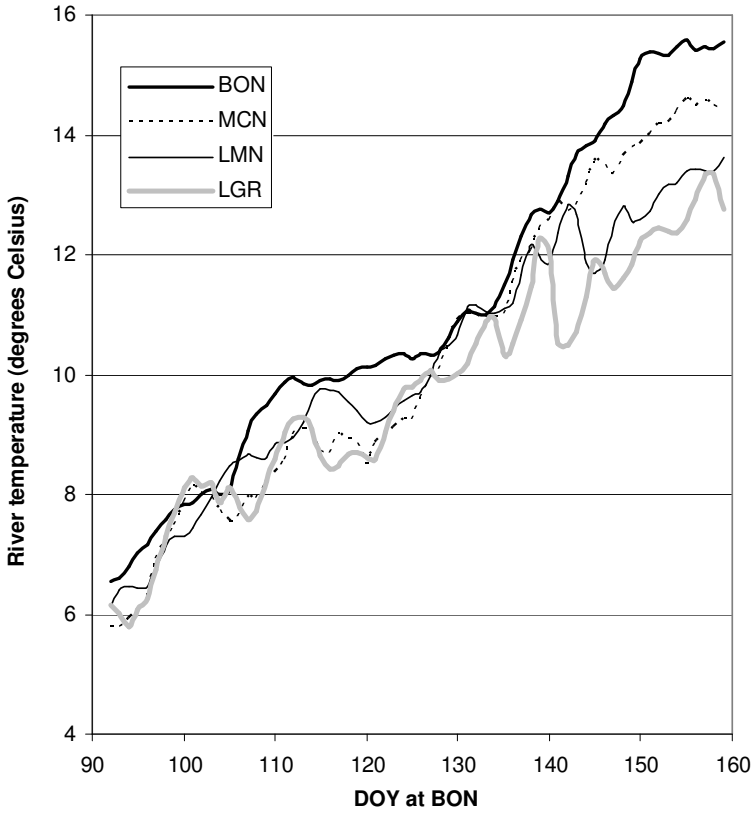

Figure A.8. River water temperature (°C) at LGR, LMN, MCN, and BON in 2009.

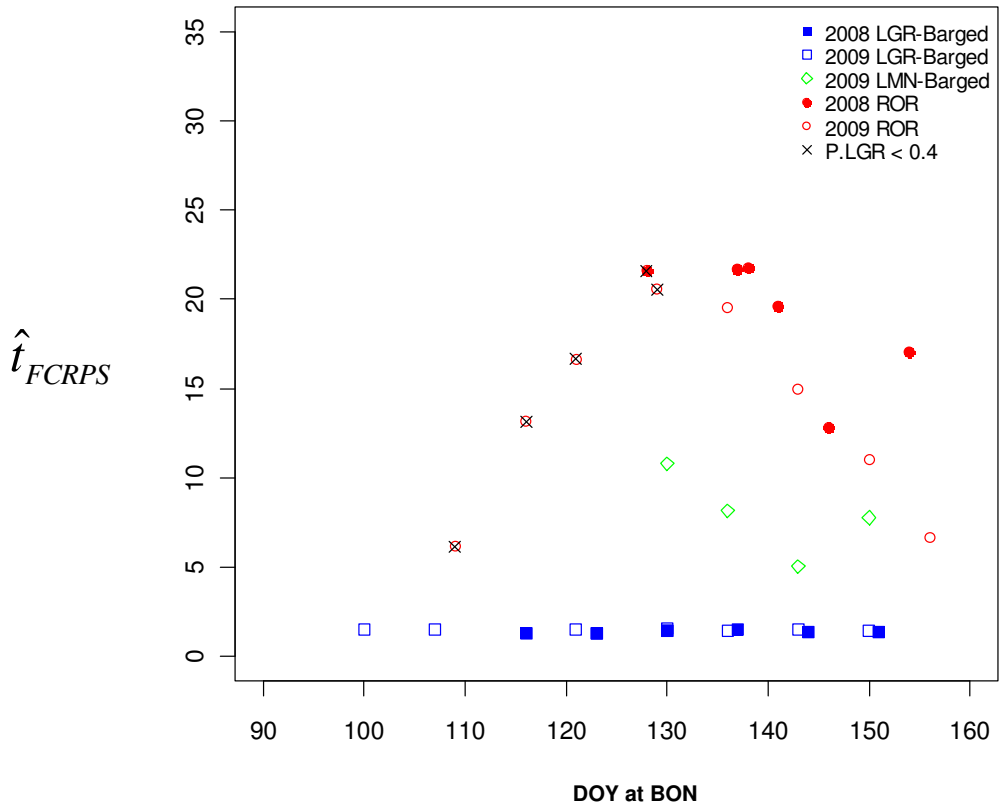

Figure A.9. Travel time ($\hat{t}_{FCRPS}$) of ROR, LGR-barged, and LMN-barged hatchery spring-summer Chinook salmon samples collected at BON. ROR fish were run-at-large, and thus their travel times were estimated with $\hat{P}_{LGR}$, $\hat{P}_{MCN}$, and $\hat{P}_{BON}$ incorporated into the estimates.

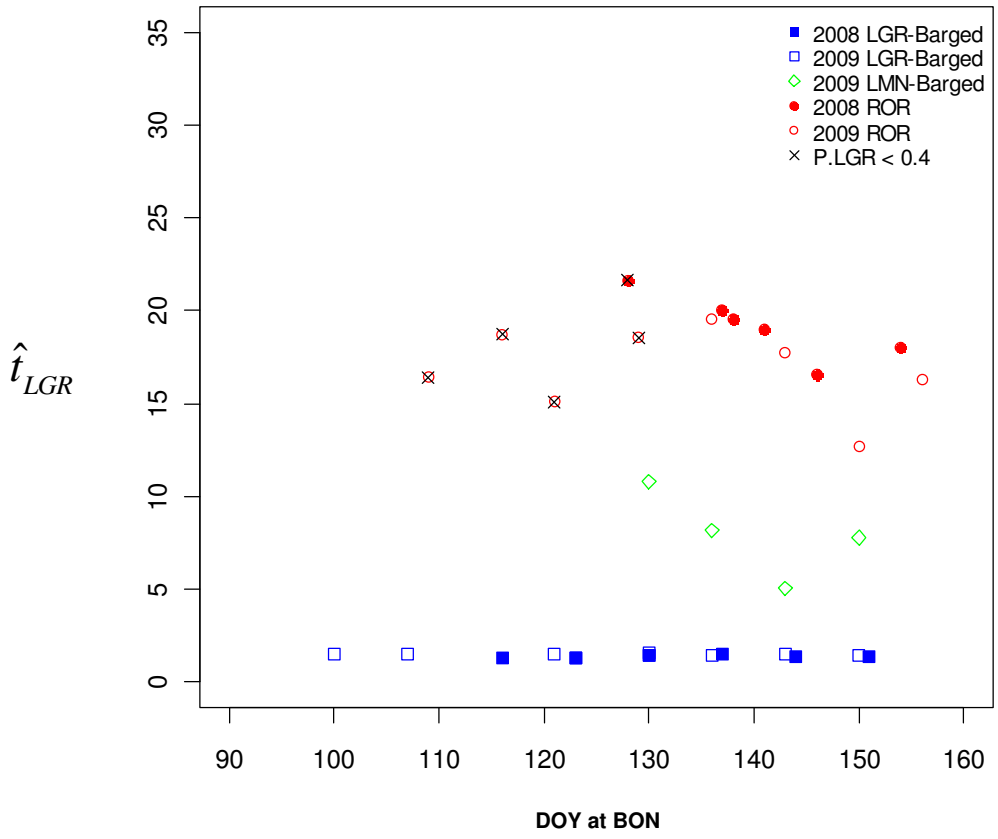

Figure A.10. Travel time ($\hat{t}_{LGR}$) of ROR, LGR-barged, and LMN-barged hatchery spring/summer Chinook salmon samples collected at BON assuming all fish originated from above LGR.

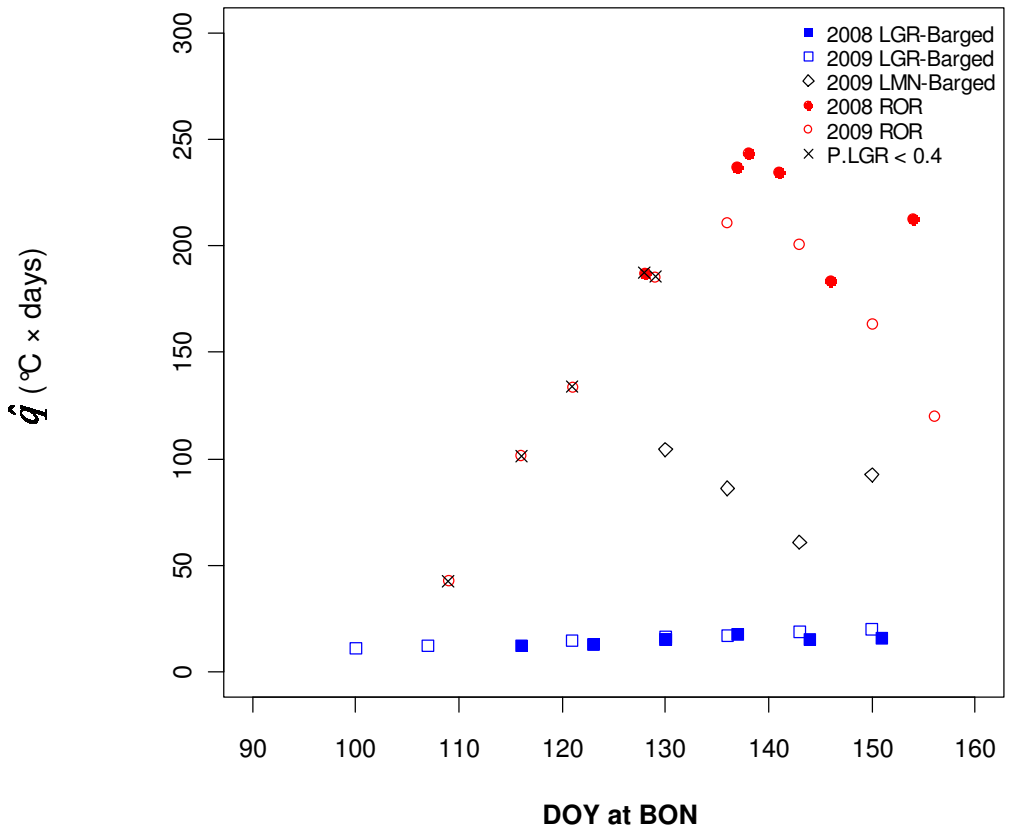

Figure A.11. Degree days $\hat{q}$ experienced by each sample of ROR, LGR-barged, and LMN-barged samples collected at BON.

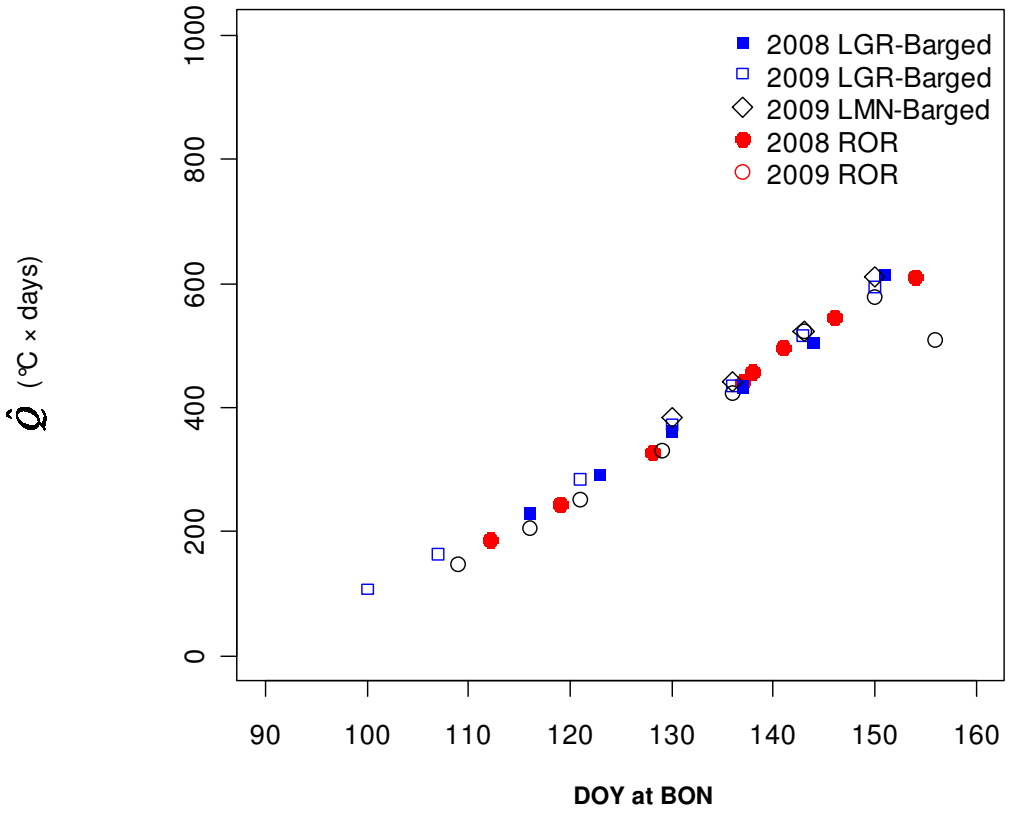

Figure A.12**.** Degree days $\hat{Q}$ experienced by each sample of ROR, LGR-barged, and LMN-barged samples collected at BON.

Appendix C.4. Interaction plots of survival capacity and predictor variables by barge index

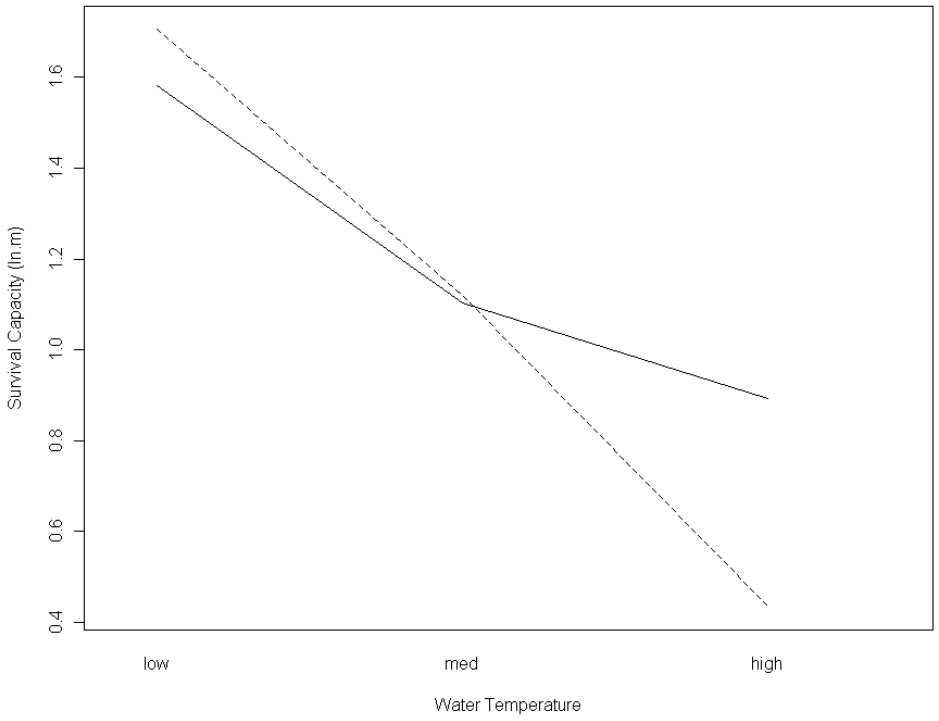

Figure A.13**.** Interaction plot between survival capacity of juvenile spring/summer Chinook salmon in challenge studies vs. water temperature T_{site} on day of sampling (low < 9.5°C, 9.5°C ≤ med < 12.5°C, high ≥ 12.5°C). Solid line represents barged fish, and dashed line represents ROR fish.

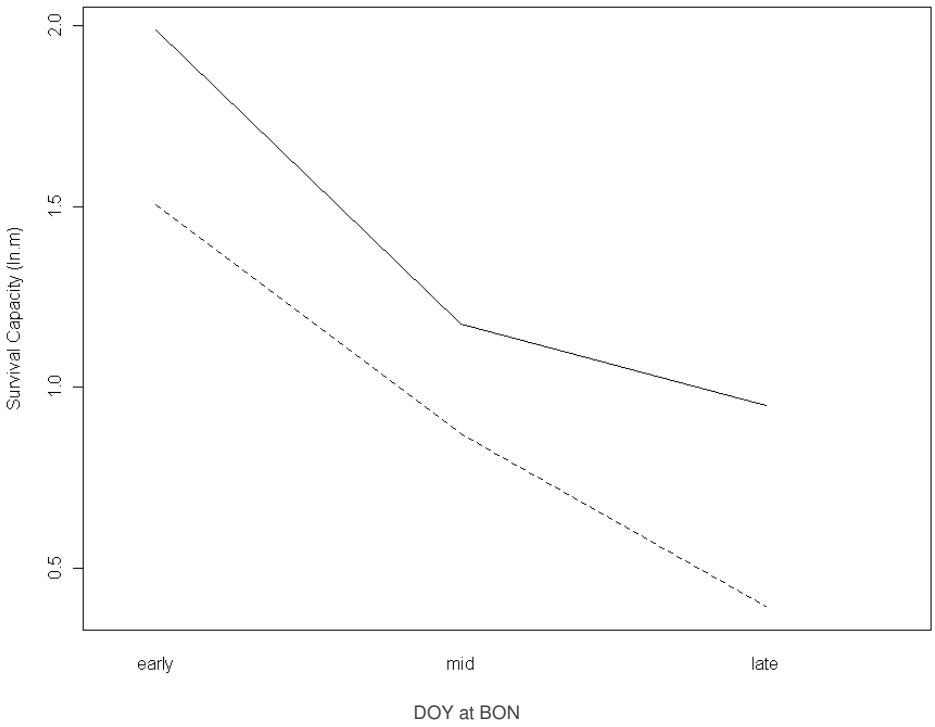

Figure A.14**.** Interaction plot between survival capacity of juvenile spring/summer Chinook salmon in challenge studies vs. DOY at BON (early < 120, 120 ≤ mid < 140, late ≥ 140). Solid line represents barged fish, and dashed line represents ROR fish.

APPENDIX D

Hypothesized Processes Involved with the Rate of MS-222 Induction for Subchapter 4B

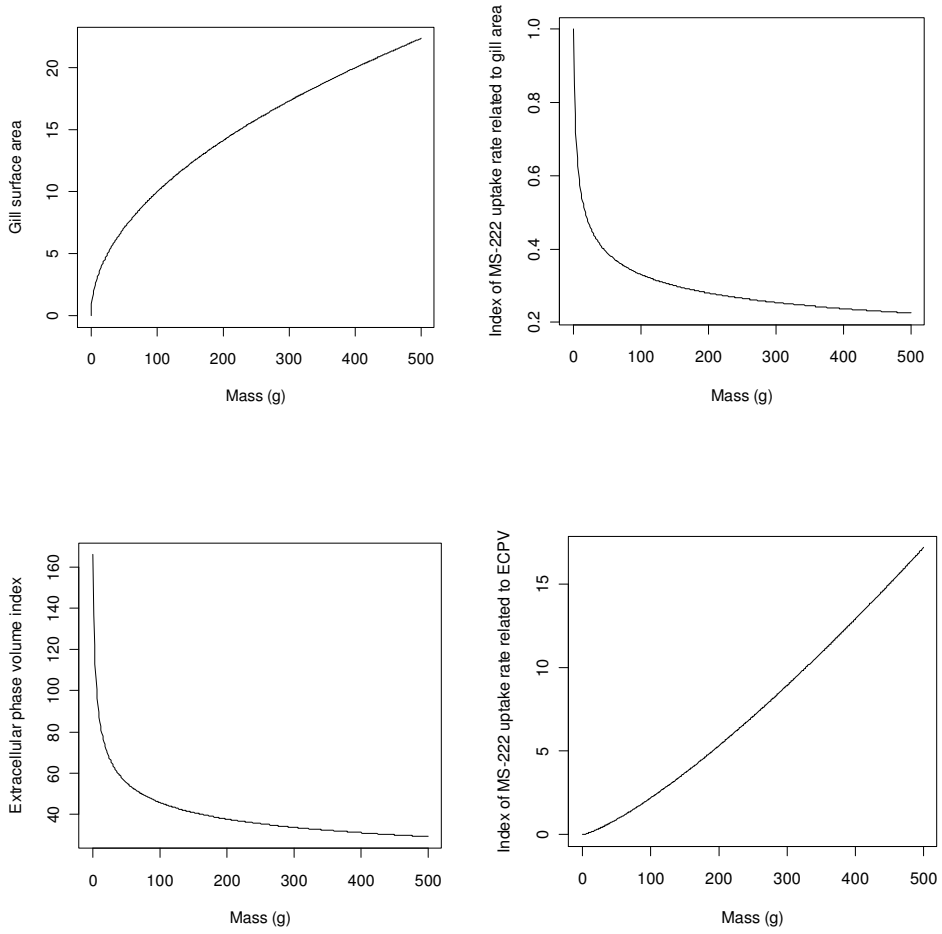

Figure A.15**.** Hypothesized processes involved with the rate of MS-222 induction. The relationship between gill surface area and body mass (gill area = mass$^{0.5}$) was chosen to be within range published by Palzenberger and Pohla (1992), and the relationship between extracellular phase volume (ECPV) and body mass [ECPV in ml/kg = 166 × (mass in g)$^{-0.28}$] was from Houston et al. (1976). As the gill surface area to mass ratio decreases, the rate of MS-222 uptake through the gills is hypothesized to decrease. As the ECPV to mass ratio decreases, the rate of MS-222 uptake through the nerve cell membrane is hypothesized to increase.

APPENDIX E

Frequency Distributions and Fitted Inverse Gaussian Distributions for Each Subsample of Each Fish Condition Index of Coho Salmon for Chapter 5

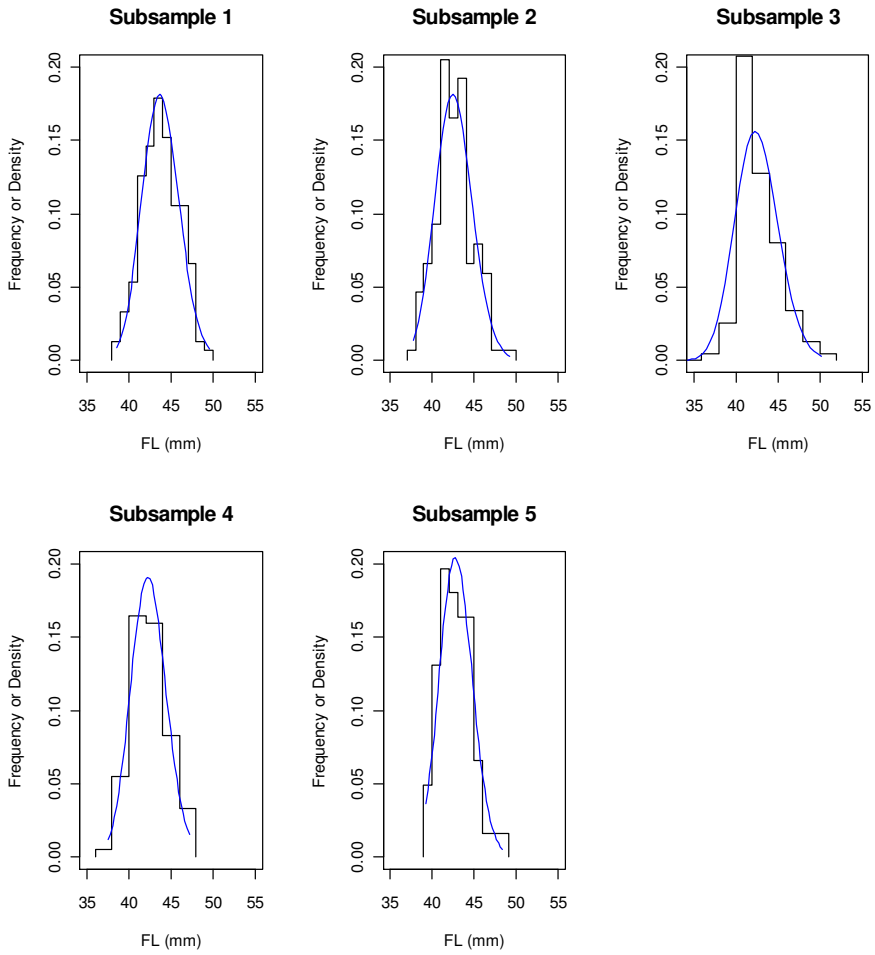

Figure A.16. Fork length: Frequency (histogram) and fitted Inverse Gaussian distribution (curve).

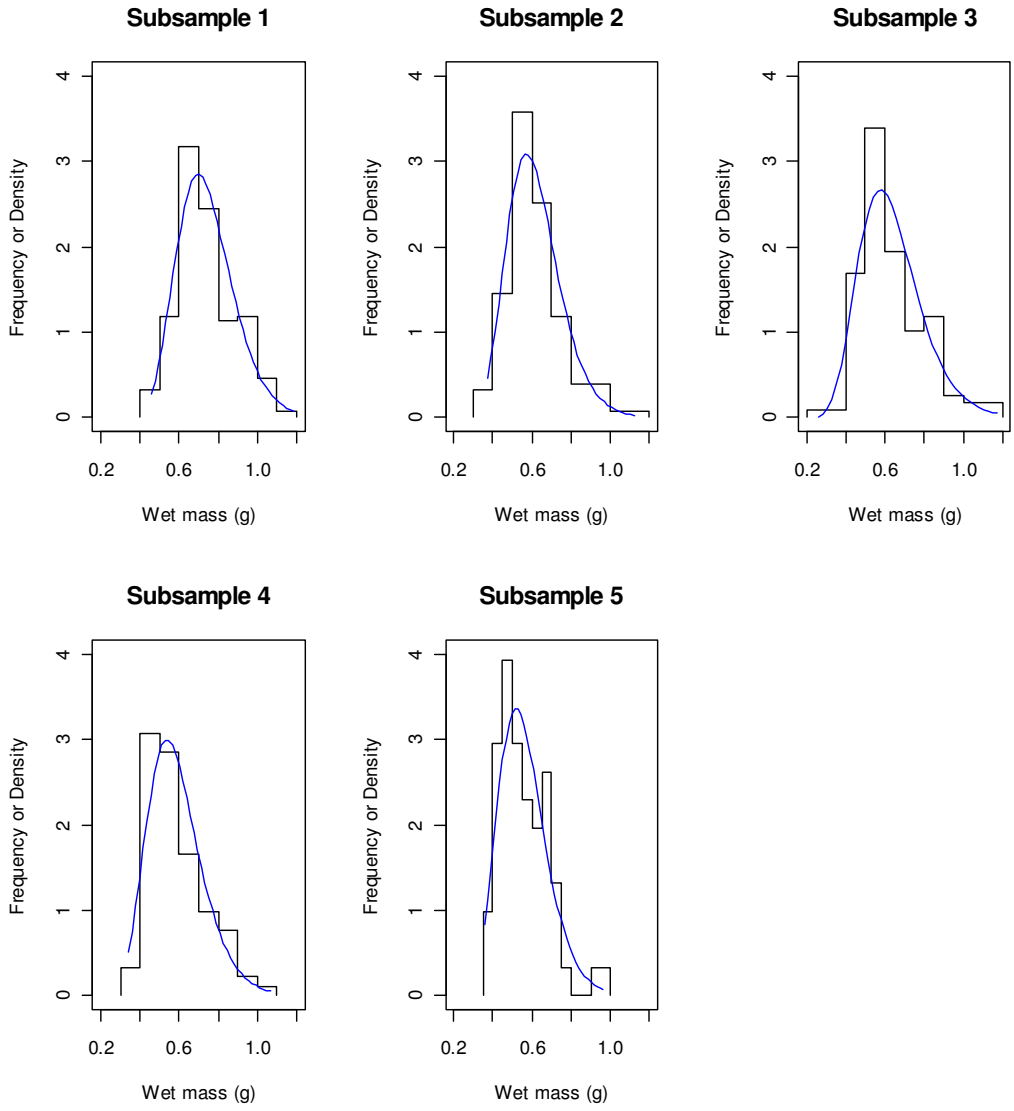

Figure A.17. Wet mass: Frequency (histogram) and fitted Inverse Gaussian distribution (curve).

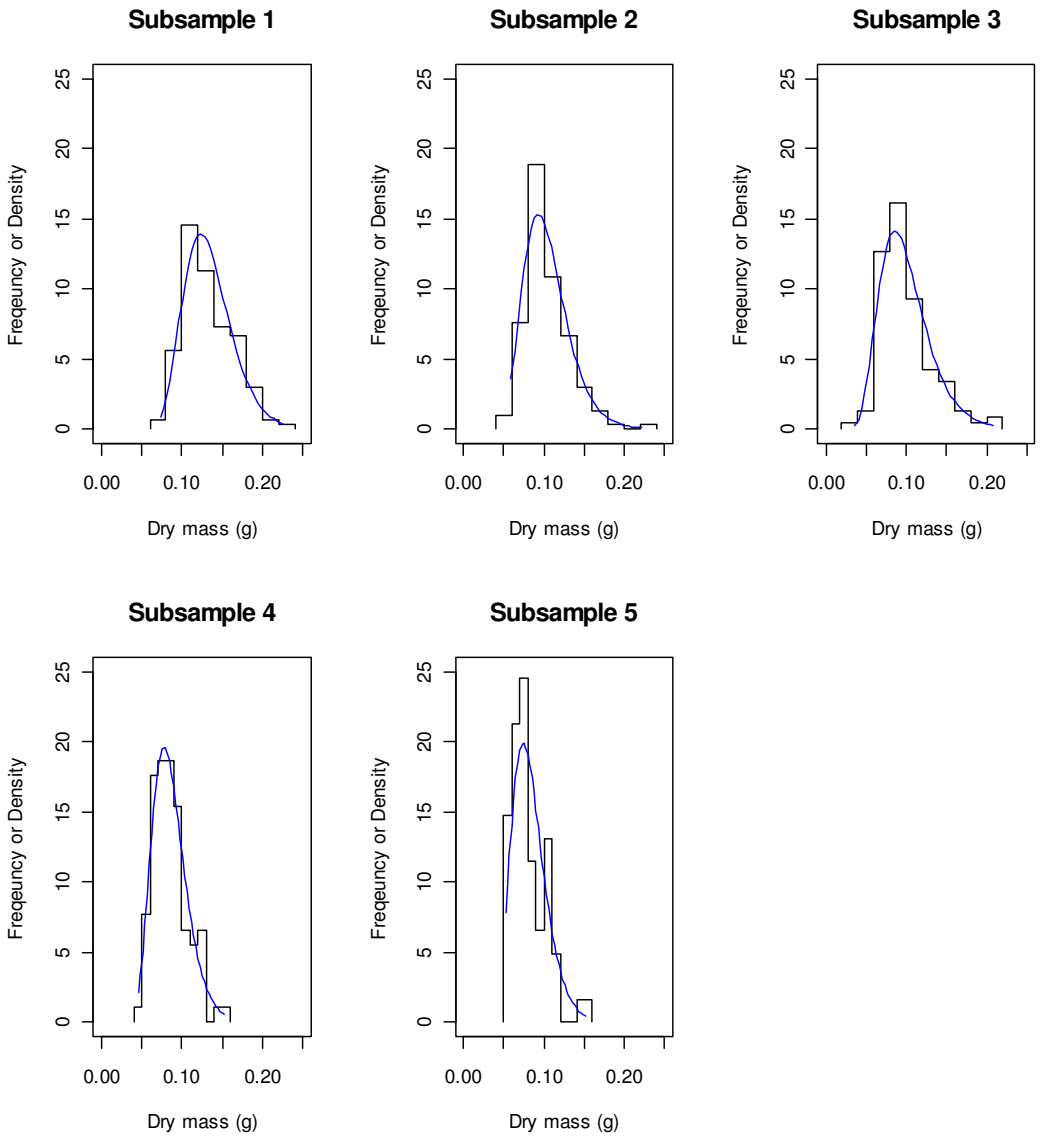

Figure A.18. Dry mass: Frequency (histogram) and fitted Inverse Gaussian distribution (curve).

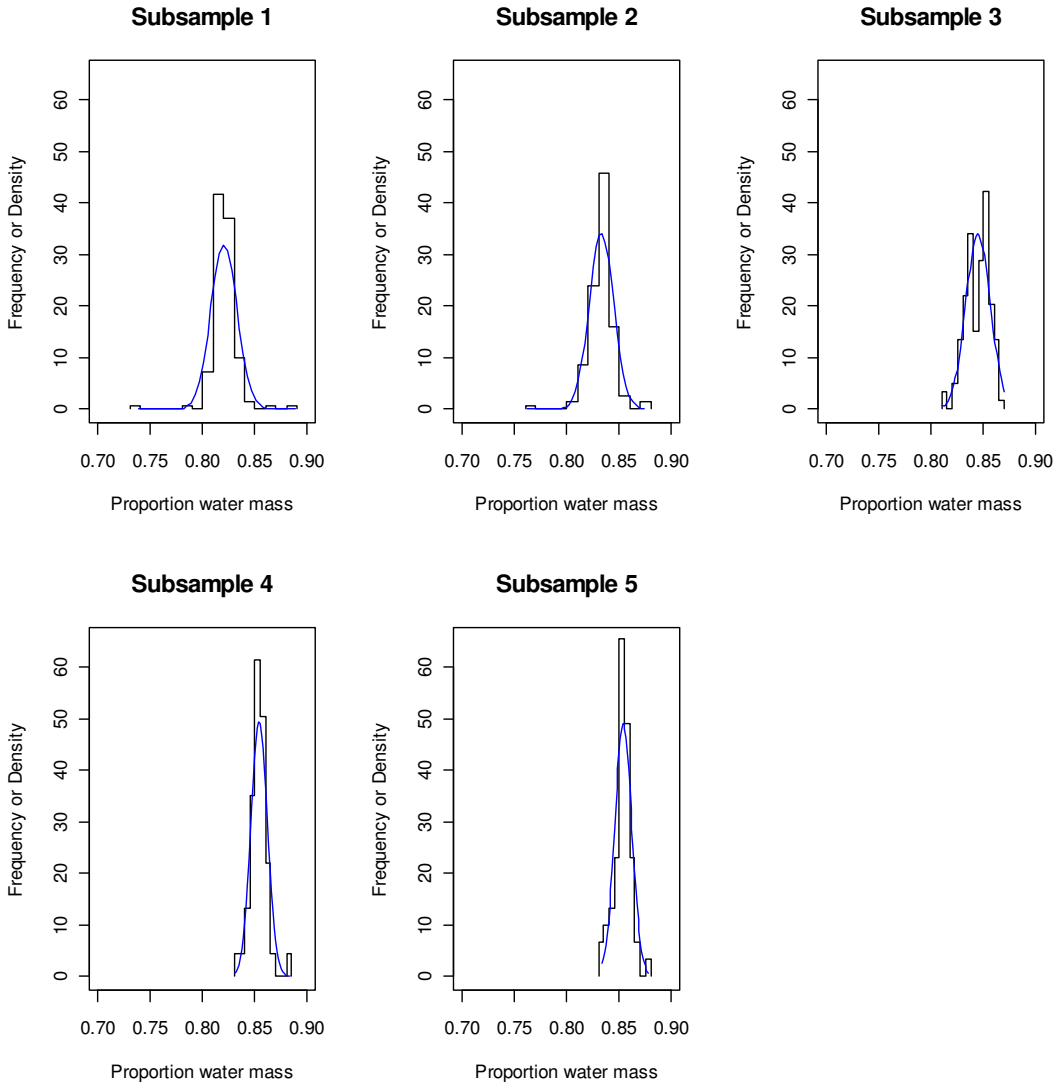

Figure A.19. Proportion water mass: Frequency (histogram) and fitted Inverse Gaussian distribution (curve).

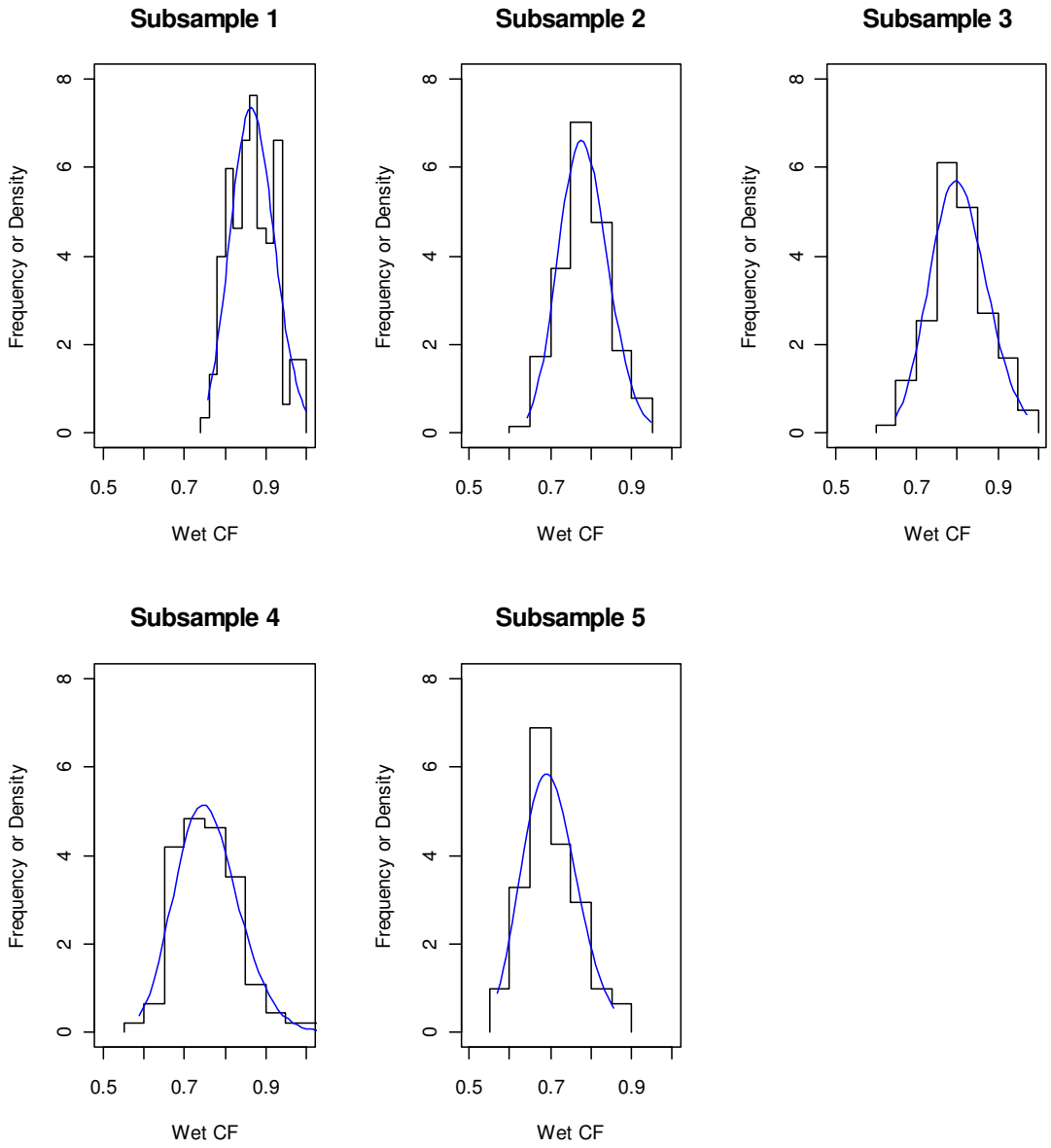

Figure A.20. Wet condition factor (CF): Frequency (histogram) and fitted Inverse Gaussian distribution (curve).

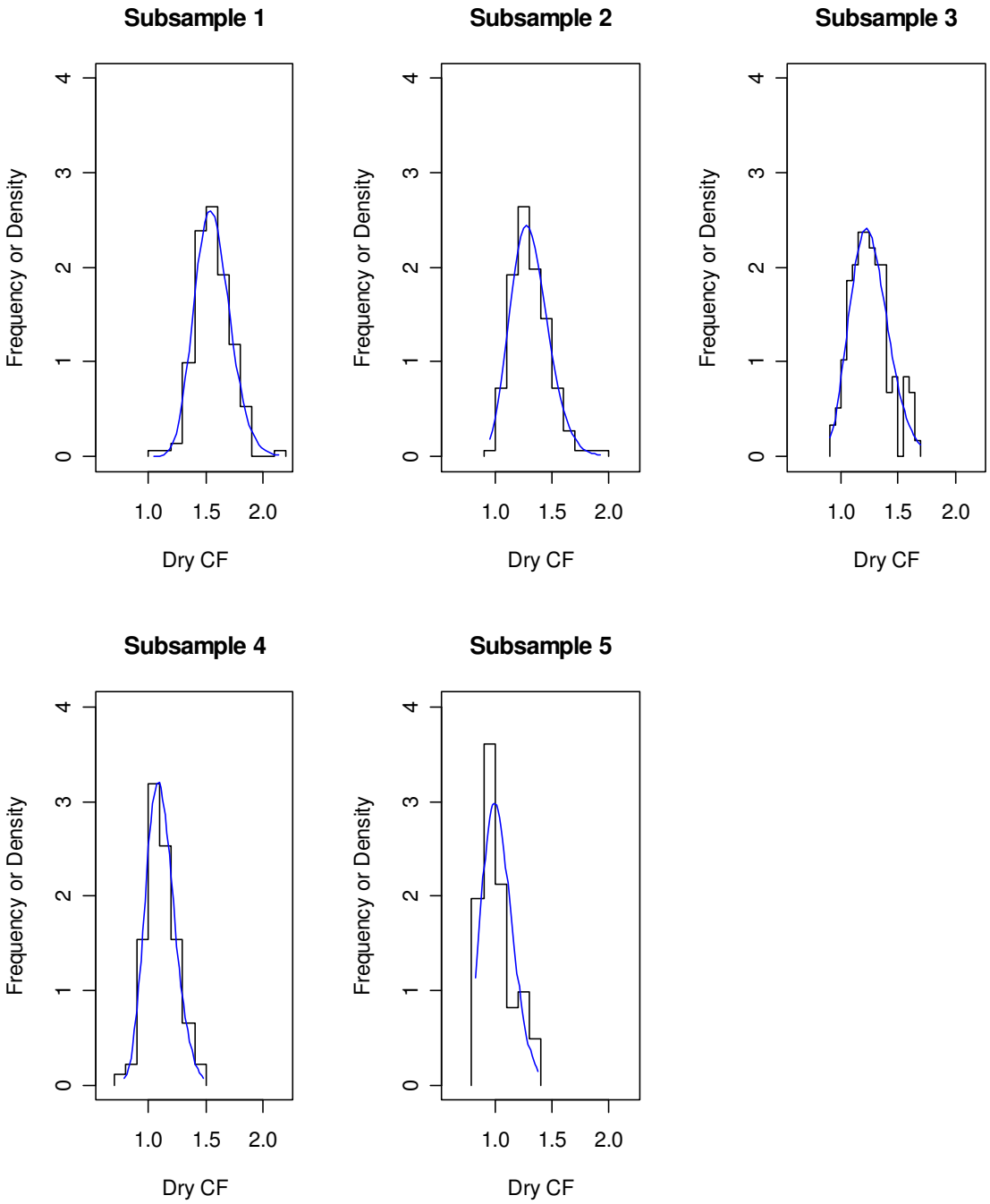

Figure A.21. Dry condition factor (CF): Frequency (histogram) and fitted Inverse Gaussian distribution (curve).

VITA

Jennifer Lam-Anh Gosselin (nee Tran) was born in Toronto, Ontario, Canada. She spent much of her childhood years in Quebec, Quebec, before returning to Toronto where she completed her high school degree with the International Baccalaureate Program at Le Collège français. Since she was 6 years old, she was interested in nature. Her love for ecology and animal behavior flourished during her studies at the University of Toronto where she earned her Honours Bachelor of Arts and Science, and at the University of Toronto at Mississauga where she earned her Master's in Biology. Now that her doctoral *life stage* has come to an end with a Doctor of Philosophy in Aquatic and Fishery Sciences from the University of Washington, she eagerly begins another *life stage*. She fondly looks back on her *cumulative experiences* and how they have positively affected her *survival capacity* on both academic and personal levels. One might even say that the stressors in this *challenge test* has made her stronger.